

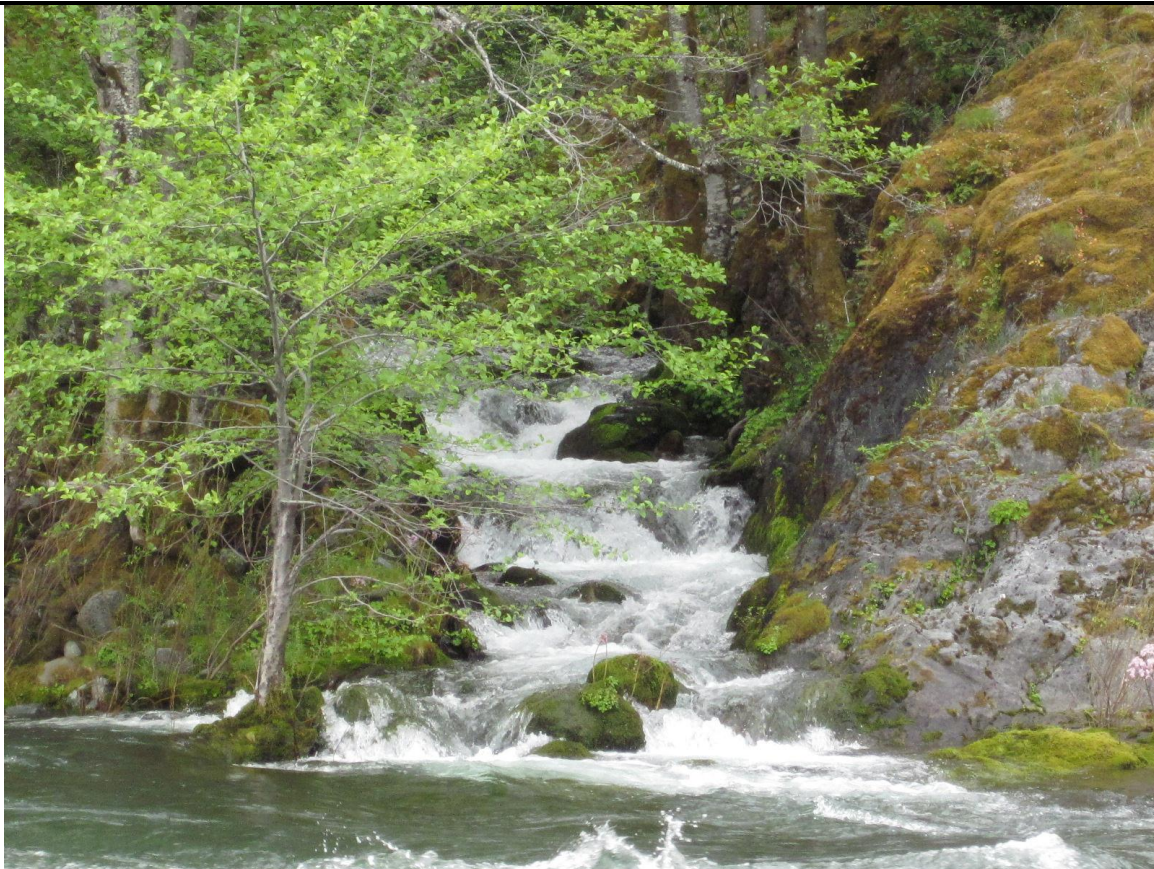
California-Nevada Fish Health Center

FY 2012 Investigational Report:

**Myxosporean Parasite (*Ceratomyxa shasta* and *Parvicapsula minibicornis*) Prevalence of Infection in Klamath River Basin Juvenile Chinook Salmon, April-August 2012**

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## SUMMARY

Juvenile Klamath River Chinook salmon (*Oncorhynchus tshawytscha*) were assayed from April to August 2012 by quantitative polymerase chain reaction (QPCR) and histology for myxosporean parasite infections, *Ceratomyxa shasta* and *Parvicapsula minibicornis*. The seasonal prevalence of *C. shasta* by QPCR in Chinook salmon collected above the Trinity River confluence during the peak migration period (May-July) was 30%, an increase from 17% observed in 2011. *Parvicapsula minibicornis* in Chinook salmon above the Trinity River confluence for the same period was 69% compared to 48% in 2011. The prevalence of *C. shasta* below the Trinity River was 54% by QPCR for fish collected later in the season (June-August) in the lower basin. *Parvicapsula minibicornis* prevalence of infection by QPCR below the Trinity River confluence for the same period was 88%.

In Iron Gate Hatchery (IGH) coded-wire tagged (CWT) juvenile Chinook salmon screened by QPCR, *C. shasta* was detected in 42% (129/306) of fish examined. The highest *C. shasta* prevalence of infection (POI) observed was 72% in the IGH-CWT Chinook salmon residing 3 Weeks at Liberty (WAL) at time of capture. Iron Gate Hatchery CWT Chinook also had much higher parasite loads in 2012 compared to 2011. *Ceratomyxa shasta* was detected in 43% (23/54) of marked Trinity River Hatchery (TRH CWT) Chinook salmon sampled in the Klamath River. *Ceratomyxa shasta* prevalence of infection was low in TRH-CWT Chinook collected in the Trinity River (7%), indicating that fish are exposed and lightly infected with *C. shasta* prior to entering the Klamath River.

In summary, both *C. shasta* prevalence of infection by QPCR in mixed origin fish collected during peak migration (30%) and in IGH CWT Chinook salmon (42%) indicate that infectivity increased in 2012 compared to 2011. Environmentally, 2012 consisted of a relatively normal temperature profile for the Klamath River. No manipulated pulse flow from Iron Gate Dam (as in 2011) or extended period of precipitation (as in 2010) occurred. The typically warm river temperatures (15-24°C) observed in May – July, coupled with earlier high *C. shasta* actinospore densities (May versus June in 2011) in the infectious zone, resulted in an increase in annual infection prevalence compared to the previous monitoring year. Historically, the 2012 annual infection prevalence for juvenile Chinook salmon during outmigration was relatively moderate compared to historical levels observed for the monitoring program (2006-2011).

The correct citation for this report is:

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### Notice

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## INTRODUCTION

Two myxozoan parasites, *Ceratomyxa shasta* and *Parvicapsula minibicornis*, share vertebrate (salmonid) and invertebrate (*Manayunkia speciosa*) hosts and have overlapping distributions throughout the Pacific Northwest (Ching and Munday 1984; Hoffmaster et al. 1988; Hendrickson et al. 1989; Bartholomew et al. 1997; Kent et al. 1997; Jones et al. 2004; Bartholomew et al. 2006, Stocking et al. 2006). *Ceratomyxa shasta* and *P. minibicornis* are distributed throughout the mainstem Klamath River system including the lower reaches of the Williamson and Sprague Rivers, Agency Lake, Klamath Lake, Copco Reservoir, and the Klamath River from Iron Gate Dam to the estuary (Hendrickson et al. 1989; Stocking et al. 2006; Bartholomew et al. 2007; Stocking and Bartholomew 2007). The polychaete worm, *Manayunkia speciosa*, is most abundant in the Klamath River in an area approximately 60 river miles (RM) extending below Iron Gate Dam to Seiad Valley. *Manayunkia speciosa* populations release an infective actinospore stage into the water column, where the parasite infects juvenile Chinook salmon (*Oncorhynchus tshawytscha*) that are rearing in, or emigrating through, this section of the mid Klamath River termed the infectious zone. Klamath River Chinook salmon can experience high prevalence and severity of infection with these two myxosporean parasites, particularly when river temperatures promote early proliferation and maturation of polychaete populations (Bartholomew & Foott 2010, True et al. 2011). Ceratomyxosis in juvenile Chinook salmon can cause significant mortality (40-50%) when river temperatures reach 15-18°C in late spring and are coupled with low flows that favor polychaete worm proliferation and maturation. Warmer temperatures also hasten disease progression within the fish host when other factors are constant (Udey 1975, Bartholomew & Foott 2010).

An annual metric for *C. shasta* prevalence of infection by histology (above the Trinity confluence and during the peak migration period of May to July) and concurrent QPCR screening is used to provide year to year comparisons of infection prevalence. Infection prevalence by histology has ranged from 15-54% and 17-49% by QPCR in study years 2006-2011 (Nichols & True 2007, Nichols et. al. 2008, Nichols et. al 2009, True et. al. 2011, and Bolick et al. 2012)(Table 5, page 28). In a typical year, *P. minibicornis* prevalence of infection can be quite high, with infection prevalence rapidly rising to 100% by May or June. Seasonal *P. minibicornis* prevalence has ranged from 48-91% in the same 2006-2011 time period (data not shown in Table 5). While the majority of fish are dual infected with both parasites, *C. shasta* is the most significant parasite in terms of clinical disease and associated mortality observed in both natural and hatchery origin Chinook salmon in the Klamath River.

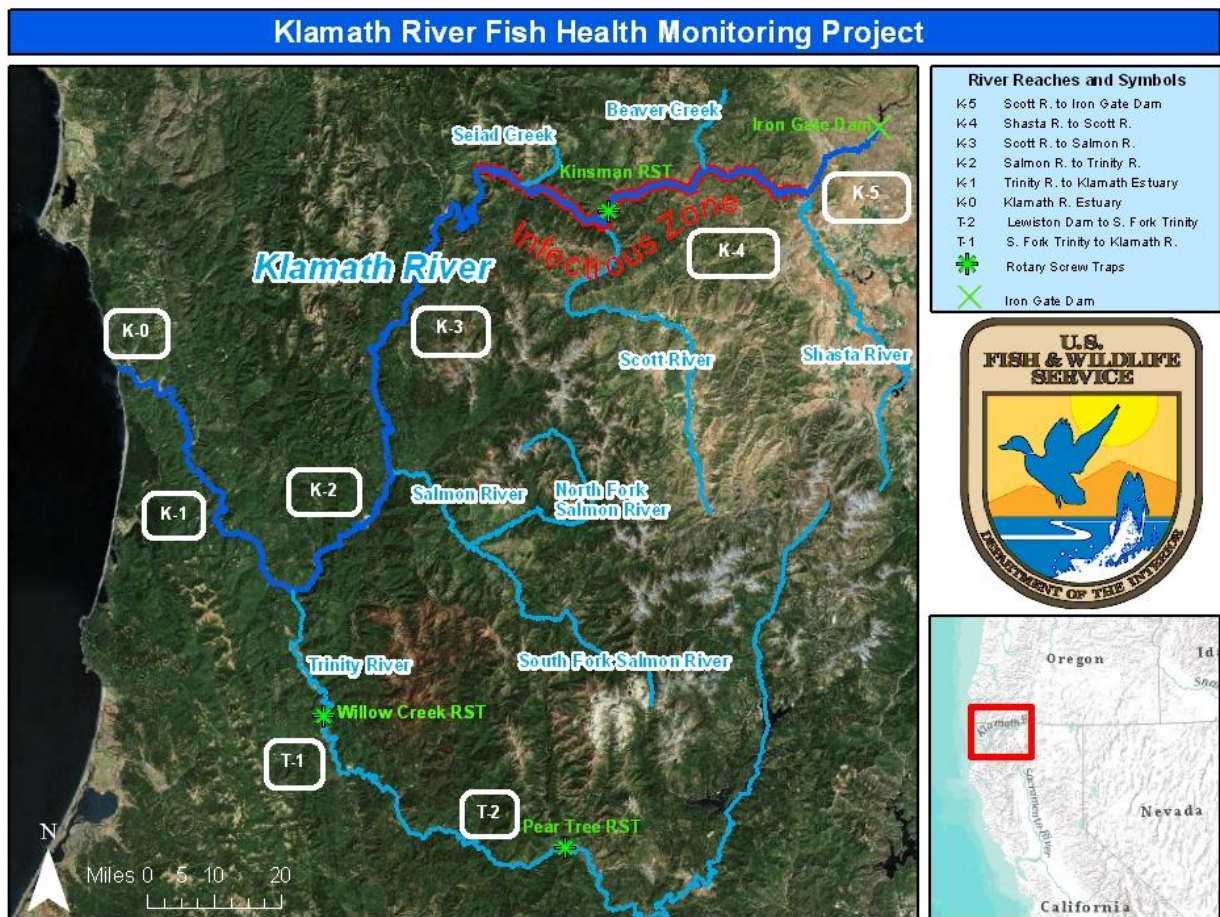
The objectives of this study were: 1) examine *C. shasta* and *P. minibicornis* prevalence in Iron Gate Hatchery (IGH) and Trinity River Hatchery (TRH) Chinook salmon prior to and post hatchery release; 2) examine the parasite prevalence in the juvenile Chinook salmon population by QPCR within specific river reaches and throughout the spring out-migration period; 3) compare parasite prevalence by QPCR in 2012 to previous years; and 4) examine the diagnostic prevalence of other significant pathogens in juvenile Chinook salmon, if they occur, by histology.



## METHODS

### Sample Sites, Fish Groups and Number Sampled

Fish were collected in the Klamath River from below Iron Gate Dam (Klamath RM 190) to the Klamath River Estuary and on the Trinity River between Lewiston Dam (Trinity RM 111) and the Trinity River confluence with the Klamath River (Klamath RM 43). Klamath and Trinity Rivers were divided into sample reaches at major tributaries, with study cooperators collecting fish in each reach (Figure 1, Table 1). When possible, existing salmonid downstream migrant trapping sites were utilized for collection, but beach seining was required to achieve the desired sample size in some weeks.



**Figure 1. Klamath River watershed, major tributaries, and sample reaches: Iron Gate dam to Shasta River (K5), Shasta River to Scott River (K4), Scott River to Salmon River (K3), Salmon River to Trinity River confluence (K2), Trinity River to Estuary (K1), Klamath River Estuary (K0). Trinity River sites: Pear Tree (T2) and Willow Creek (T1) rotary screw traps.**

**Table 1. Sample reach locations (reach code), river mile, and cooperating agencies performing fish collections on the Klamath and Trinity rivers.**

<b>Sample Reach (reach code)</b>	<b>River Mile</b>	<b>Primary collector(s)</b>
<b>Klamath River main stem</b>		
Shasta to Scott (K4)	Klamath 177-144	USFWS and Karuk Tribe
Scott to Salmon (K3)	Klamath 144-66	Karuk Tribe
Salmon to Trinity (K2)	Klamath 66-44	Karuk Tribe
Trinity to Estuary (K1)	Klamath 44-4	Yurok Tribe
Klamath Estuary (K0)	Klamath 4-0	Yurok Tribe
<b>Trinity River</b>		
Upper – Pear Tree Rotary Trap (T2)	Trinity 94	Hoopa Valley Tribe
Lower - Willow Creek Rotary Trap (T1)	Trinity 21	Yurok Tribe

All fish collected from the two rivers were categorized into three sample group types based on their origin: natural, unknown and coded wire tagged. “Mixed origin” Chinook refers to any or all group types as a whole, i.e., results for all fish collected in a particular reach, or for the entire sampling season. The sample group types are defined below.

Natural: Chinook salmon collected early in the sampling season (April-May) prior to hatchery smolt releases.

Unknown: Unmarked Chinook salmon collected after hatchery release, undifferentiated fish could be natural or hatchery origin.

Coded wire tagged (CWT): Hatchery Chinook marked with an adipose fin clip and implanted with a coded wire tag before release.

Additionally, a pre-release exam of hatchery Chinook salmon for *C. shasta* and *P. minibicornis* infections was performed before fish were released: 29 were sampled from IGH on May 17, and 30 fish were sampled from TRH on May 16. Iron Gate Hatchery releases approximately 5 million fall Chinook salmon in late May to early June: in 2012 the releases occurred from June 6<sup>th</sup>-18<sup>th</sup>. Trinity River Hatchery volitionally releases approximately 1.5 million fall Chinook and approximately 1.3 million spring Chinook salmon each year: in 2012 this occurred from June 1-15<sup>th</sup>. After hatchery Chinook were released, the study focused on capture and testing of CWT fish, to assess parasite loads in relationship to Weeks At Large (WAL).

Fish numbers tested in the Klamath River varied by reach, with emphasis on natural fish in the reaches below Iron Gate Dam initially, then hatchery CWT fish for the remainder of the spring/summer migration period (Table 2). In the Trinity River, we sampled the upper and lower watershed at rotary screw traps (RST) located at Pear Tree and Willow Creek out migrant monitoring sites. Similar to the Klamath River sampling, effort focused on natural fish initially, then CWT marked hatchery Chinook salmon as they emigrated through the lower Klamath reaches, and finally in the Klamath River Estuary (Table 2).

**Table 2. Number of fish sampled for QPCR testing by Klamath River reach (reach code) and sampling week. Supplemental samples for histology (H) were collected in four reaches.**

Week	Sample Date	Shasta R. to Scott R. (K4)	Scott R. to Salmon R. (K3)	Salmon R. to Trinity R. (K2)	Trinity R. to Estuary (K1)	Klamath R. Estuary (K0)	Upper Trinity R. (T2)	Lower Trinity R. (T1)
1	1-Apr							
2	8-Apr	15						
3	15-Apr	15						
4	22-Apr	15						
5	29-Apr	15 (H10)	15	15	15		15	15
6	6-May	15						
7	13-May	15 (H9)	15	15	15		15	15
8	20-May	15						
9	27-May	15 (H10)	15	15	15		15 (H9)	15 (H10)
10	3-Jun	14						
11	10-Jun	20 (H10)	20	20				
12	17-Jun	20	21 (H10)	21 (H10)	20 (H10)	3	20	20
13	24-Jun	20 (H10)	20	20		20		
14	1-Jul	20	40 (H10)	20 (H10)	21 (H10)	20	12 (H10)	18 (H7)
15	8-Jul	11	44	20		20		
16	15-Jul		20 (H10)	20 (H10)	20 (H10)	20	20	20
17	22-Jul					20		
18	29-Jul				20 (H10)	20	20	17
19	5-Aug					20		
20	12-Aug				20	20		
21	19-Aug					20		

Heads from all CWT marked IGH or TRH Chinook salmon collected were tracked with unique identification numbers and CWT codes were read by the USFWS Arcata Fish and Wildlife Office (AFWO). The date each group of CWT Chinook salmon was released from the hatchery and date of recapture was used to assess temporal infections levels in individual fish by comparing parasite load to WAL. Releases at TRH were volitional and occurred from June 1 through June 15 (June 8 was used as the mean date of release) and IGH released Chinook on June 6 through June 18 (specific release dates were provided for given tag codes).

#### Parasite Infection Levels by Quantitative PCR Assays

Fish to be tested by QPCR were euthanized, placed in a plastic bag labeled with date and reach, and frozen in the field. In the laboratory, fish were thawed, fork length was measured, clinical disease signs notated, and necropsy performed to collect intestine and kidney tissues for *C. shasta* and *P. minibicornis* testing, respectively. The entire intestine and kidney from each fish were removed and combined into a single well of a 96 well plate. Tissue samples were then frozen at -20 °C until DNA extraction was performed.

Combined intestine and kidney tissues were digested overnight in 100µL MagMAX Proteinase K Buffer containing 100 mg/ml proteinase K (Applied Biosystems, Foster City, CA) at 55°C with constant shaking. A subsample of digested tissue homogenate was diluted 1:10 in molecular grade water, then diluted 1:10 in MagMAX Multi-Sample DNA Lysis Buffer (Applied Biosystems, Foster City, CA) for a final dilution of 1:100. The diluted tissue homogenate was extracted in a 96 well magnetic bead sample processing system (Applied Biosystems MagMAX Express-96 Magnetic Particle Processor). Extracted DNA was stored at -20 °C until the QPCR assays were performed.

Samples were assayed in Real Time PCR Sequence Detection Systems (SDS) using probes and primers specific to each parasite. The combined tissues were tested for *C. shasta* 18S rDNA using TaqMan Fam-Tamra probe and primers (Hallett and Bartholomew 2006) on the 7300 Sequence Detection System (Applied Biosystems, Foster City, CA). Separately, the combined tissues were tested for *P. minibicornis* 18S rDNA utilizing TaqMan Minor-Groove-Binding (MGB) probe and primers (True et al. 2009) on the StepOne Plus Sequence Detection System or the 7300 Sequence Detection System (Applied Biosystems Foster City, CA). Prevalence of *P. minibicornis* infection is generally high early in the season, and remains high throughout the migration period. Therefore, kidney tissue for *P. minibicornis* testing was collected from all fish, however only representative sub-sets of samples from each reach were assayed by QPCR. Reaction volumes of 30µL, containing 5µL DNA template, were used for both assays under the following amplification conditions: 50 °C for 2 min.; 95 °C for 10 min; 40 cycles of 95 °C for 15s and 60 °C for 1 min. Plasmid standards, extraction control and no template control (NTC) wells were included on each assay plate. Cycle threshold ( $C_T$ ) values were calculated using SDS software (7300 SDS v 1.3.1, StepOne SDS v. 2.0 Applied Biosystems) and a standard curve to transform  $C_T$  values to parasite DNA copy number.

### Histological Assays

Histology sampling in 2012 included four reaches on the Klamath River (K4, K3, K2, and K1), as well as the Trinity River sample locations (T2 and T1) (Table 2).

Fish to be tested by histology were euthanized and placed in Davidson's fixative within 2 minutes of euthanasia. Fish were held in fixative for 24-48 hours. The fixative was replaced with 70% ethanol for storage until the gross examination and histological processing were performed. Each histological cassette contained kidney, intestine, and 1 to 2 gill filaments. Specimens were processed for 5µm paraffin sections and stained with hematoxylin and eosin (Humason 1979). All tissues for each fish were placed on one slide and identified by a unique number code. Each slide was examined at 40X to 400X magnification.

Histological rankings of 'clinical disease' included a pathology score: a numeric index of disease severity for kidney and intestine. The pathology score does not affect the overall prevalence of infection reported for histological assessments, but provides a numeric index of the disease state in sample groups. Pathology score is based on the degree of specific tissue abnormalities and parasite distribution (0 = normal, 1= focal, 2 = multi-focal, and 3 = diffuse distribution, Table 3). A kidney pathology score was calculated by summing the score of each kidney lesion (interstitial hyperplasia, necrotic interstitium or tubule, interstitial granuloma, glomerulonephritis, and protein casts within the glomeruli or tubules). The mean kidney pathology score was reported for each collection group to demonstrate severity of disease. Similarly for the intestine, the sum of lesion scores (lamina propria hyperplasia, necrotic epithelium / sloughing, necrotic muscularis) was used to calculate a collection group's mean intestinal pathology score.

**Table 3. Parasite abbreviations and tissue abnormalities listed in the histological result tables.**

<b>Kidney</b>  <i>P. minibicornis</i> Troph. <i>P. minibicornis</i> Myxosp. Metacercaria <i>C. shasta</i> troph. Chloromyxum sp  <b>Pathology Score</b>	<i>Parvicapsula minibicornis</i> trophozoite stage <i>Parvicapsula minibicornis</i> myxospore stage Immature trematode stage <i>Ceratomyxa shasta</i> trophozoite stage Chloromyxum species trophozoite stage  <b>Mean kidney pathology score for sample group</b>
<b>Intestine</b>  <i>C. shasta</i> troph. <i>C. shasta</i> myxosp. Helminth  <b>Pathology Score</b>	<i>Ceratomyxa shasta</i> trophozoite stage <i>Ceratomyxa shasta</i> myxospore stage Trematode, nematode, or cestode  <b>Mean intestine pathology score for sample group</b>
<b>Gill</b>  Ich Glochidia Metacercaria Invasive <i>C. shasta</i> Amoeba Multif. Hyperplasia	<i>Ichthyophthirius multifiliis</i> Larval mussel stage within lamellae Immature trematode stage Single cell trophozoite-like stage Amoeba associated with lamellae Multifocal hyperplastic regions on lamellae
<b>Other</b> Adipose steatitis Adipose lipofuscin	Inflammation of visceral fat tissue Oxidized lipopigments within adipose cells

#### Statistical Analysis and Terms Used

Prevalence of infection and annual prevalence (defined below) for *C. shasta* and *P. minibicornis* are reported with 95% confidence intervals (denoted ci) for each sample reach. Prevalence of infection is used to describe ratios of infected Chinook salmon (numerator) in the sample (number of animals examined) for a calendar week. Annual prevalence is used to describe the overall prevalence of infection for the sampled population during the sampling period that year. Definitions of the two terms used are as follows (Durfee 1978, USFWS Fish Health Policy FW713):

Prevalence of infection (also referred to point prevalence, abbreviated POI): Number of cases of a disease which are detected in a population *at a designated point in time*. This is usually expressed as a ratio where the numerator is the number of cases detected at a point in time and the denominator is the sample from a population from which the cases were drawn.

Annual prevalence (also referred to as period prevalence, abbreviated APOI): Measures the total number of cases known to occur during a given period. Period prevalence is often mislabeled as incidence data because the factor time enters into it. However, it should be noted that incidence describes only *new cases in a specified population*, and *requires knowledge of when the animals became infected* to determine the rate of infection (incidence attack rate).



## RESULTS

### PRE-RELEASE EXAMINATION OF IGH AND TRH CHINOOK SALMON

Infections of *C. shasta* or *P. minibicornis* were not detected by QPCR in IGH Chinook salmon prior to release; fish were sampled May 17 and released from June 6-18. Similarly, neither *C. shasta* nor *P. minibicornis* were detected in TRH Chinook salmon examined May 16, and released from June 1-15.

### PARASITE PREVALENCE OF INFECTION BY FISH ORIGIN

In 2012 we examined a total of 1167 Chinook salmon collected from the Klamath and Trinity Rivers, consisting of 345 natural fish, and 822 fish collected after hatchery release which included 554 CWTs. Coded wire tagged Chinook salmon accounted for 47% of all fish sampled in 2012. Natural fish account for 30%, and 23% of the fish are of unknown origin (unmarked hatchery fish or natural) (Figure 2).

#### Natural Production Chinook Salmon

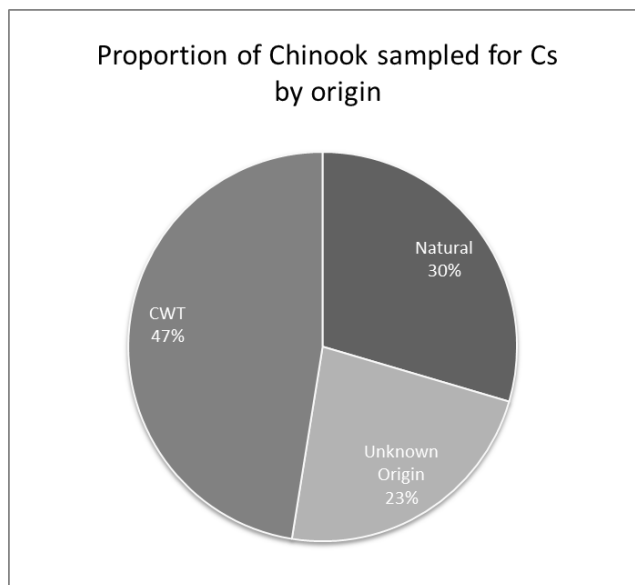
Natural Chinook salmon represent early infection status for *C. shasta* and *P. minibicornis*, as river temperatures are generally 8-10 °C cooler in the months of April and May compared to the peak hatchery salmon migration period of June-July. A total of 210 natural fish were sampled above the Trinity River confluence (K4, K3, and K2) from April 11 through May 31, and *C. shasta* was detected by QPCR in 3.8% (8/210, ci = 2-7%) compared to 2.2% (1/45, ci = 0-12%) of fish sampled below the Trinity confluence (K1 reach) and within the Estuary (K0) (Table 4).

Comparatively, *P. minibicornis* POI in natural Chinook salmon sampled above and below the Trinity confluence was 28.1% (59/210, ci = 22-35% and 26.7% (12/45, ci = 15-42%) respectively.

#### Unknown Origin Chinook Salmon

As described in the methods section, unknown origin Chinook are unmarked fish collected after hatchery release that cannot be differentiated from either natural fish or unmarked hatchery fish. A total of 225 fish of unknown origin were collected in the upper Klamath reaches from June 7 through July 20. Below the Trinity River confluence, a total of 41 fish of unknown origin were collected from the Trinity River Confluence to the Estuary (K1) from June 20 through August 14. An additional 2 fish were sampled in the Trinity River, for a total of 268 fish of unknown origin sampled in 2012.

*Ceratomyxa shasta* was detected by QPCR in 40.4% (91/225, ci = 34-47%) of Chinook sampled above the Trinity River confluence compared to 53.7% (22/41, ci = 37-69%) of fish sampled below the confluence (Table 4). *Parvicapsula minibicornis* POI in Chinook salmon of unknown origin sampled above and below the Trinity confluence was 94.7% (36/38, ci = 74-96%) and 90.5% (19/21, ci = 70-99%) respectively.



**Figure 2. Proportion and origin of Chinook salmon collected and sampled for *Ceratomyxa shasta* (Cs) testing.**

### Marked (CWT) Chinook Salmon

A total of 166 CWT Chinook was sampled in the upper Klamath reaches from June 12 through July 20. An additional 243 CWT Chinook were sampled in the lower Klamath reaches from June 20 through August 23. One hundred forty five fish were sampled in the Trinity River, for a total of 554 CWT fish sampled in 2012.

Prevalence of *C. shasta* infections in CWT Chinook salmon, sampled above the Trinity confluence was 36.7% (61/166, ci = 29-45%). Below the confluence, *C. shasta* POI was 46.9% (114/243, ci = 41-53%) (Table 4). Prevalence of *P. minibicornis* infection in CWT Chinook salmon sampled above the Trinity River confluence was 77.7% (129/166, ci = 47-59%) and 86.8% (211/243, ci = 82-91%) below the Trinity River confluence (Appendix C). Analysis of Weeks at Large data is discussed in further detail in a separate section of the report (pg. 25).

### Mixed-Origin Chinook Salmon Collected above and below the Trinity River Confluence

As described in the methods section, the mixed-origin sample group consists of natural, unmarked fish of unknown origin, and CWT Chinook salmon. A total of 601 mixed origin Chinook were sampled in the upper Klamath reaches from April 11 through July 20. A total of 329 mixed origin Chinook were sampled in the lower Klamath reaches from May 1 through August 23. An additional 237 fish were sampled in the Trinity River, for a total of 1167 fish sampled in 2012.

Prevalence of *C. shasta* infections in mixed-origin Chinook salmon, sampled above the Trinity confluence was 26.6% (160/601, ci = 23-30%) by QPCR. Below the confluence, *C. shasta* POI was 41.6% (137/329, ci = 19-26%) (Table 4). Note that in the Trinity to Estuary (K1) reach, these fish consisted of approximately equal proportions of natural fish (sampled April through May before hatchery release), mixed origin (sampled June through mid-August) and CWT Chinook salmon (sampled late June through late August). Whereas the fish sampled in the Estuary (K0) consisted of all CWT Chinook. Prevalence of *P. minibicornis* of infection in mixed origin Chinook salmon sampled above the Trinity River confluence was 51.4% (224/414, ci = 49-59%) and 78.3% (242/309, ci = 73-83%) below the Trinity River confluence.

Histological assessments were performed on random, but separate, mixed-origin fish collected from the same reaches and locations as fish tested by QPCR (Appendix A, Tables A1-A6). In 2012, poor fixation occurred in 8 of 19 (42%) collection groups and impaired evaluation of the intestine and gill tissues (body cavities were not opened prior to fixation or fish were not immediately fixed). *Ceratomyxa shasta* infection prevalence was low (15 of 150 fish) with 5 of these infections having a single trophozoite within peritoneal granuloma. No sign of intestinal invasion was seen in these fish.

**Table 4. *Ceratomyxa shasta* prevalence of infection in Chinook by fish origin (Natural, Unknown Origin, and CWT) and reach in which fish were collected in the Klamath River. Percent positive is reported with 95% confidence interval in parentheses.**

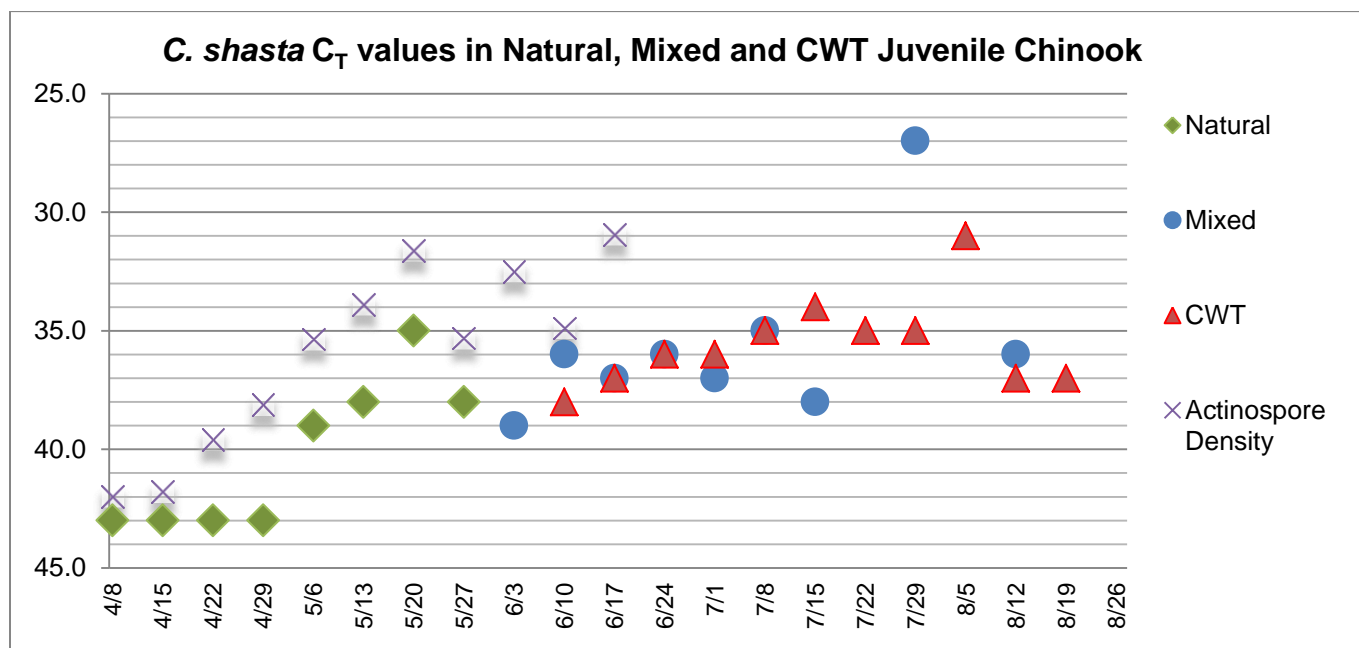
	<b>Shasta to Scott (K4)</b>	<b>Scott to Salmon (K3)</b>	<b>Salmon to TR (K2)</b>	<b>TR to Estuary (K1)</b>	<b>Estuary (K0)</b>
<b>Natural Chinook salmon – Sampled April 11 through May 31</b>					
<i>C. shasta</i> + / N	7/120	1/45	0/45	1/45	NC
<i>C. shasta</i>	<b>6% (2-12)</b>	<b>2% (0-12)</b>	<b>Negative</b>	<b>2% (0-12)</b>	NC
<b>Unknown Origin Chinook salmon (unmarked) – Sampled June 7 through August 14</b>					
<i>C. shasta</i> + / N	0/14	34/95	57/116	22/41	NC
<i>C. shasta</i>	<b>Negative</b>	<b>36% (26-46)</b>	<b>49% (40-59)</b>	<b>54% (37-69)</b>	NC
<b>CWT Chinook salmon – Sampled June 28 through August 19</b>					
<b>IGH-CWT</b> <i>C. shasta</i> + / N	11/75	37/61	4/4	33/42	44/124
<i>C. shasta</i>	<b>15% (8-25)</b>	<b>61% (47-73)</b>	<b>100%</b>	<b>79% (63-90)</b>	<b>35% (27-44)</b>
<b>TRH-CWT</b> <i>C. shasta</i> + / N	ND	ND	ND	2/5	21/49
<i>C. shasta</i>	ND	ND	ND	<b>40% (5-85)</b>	<b>43% (29-58)</b>
<b>Unknown Hatchery<sup>1</sup></b>	1/6	3/5	NC	4/5	3/5
<b>Unreadable CWT<sup>1</sup></b>	2/10	2/4	1/1	4/8	3/5
<b>ALL CWT<sup>1</sup></b> <i>C. shasta</i> + / N	14/91	42/70	5/5	43/60	71/183
<i>C. shasta</i>	<b>15% (9-24)</b>	<b>60% (48-72)</b>	<b>100%</b>	<b>72% (59-83)</b>	<b>39% (32-46)</b>
<b>Mixed Origin Chinook (Combined natural, unknown and CWT) – Sampled April 11 through August 23</b>					
<i>C. shasta</i> + / N	21/225	77/210	62/166	66/146	71/183
<i>C. shasta</i>	<b>9% (6-14)</b>	<b>37% (30-44)</b>	<b>37% (30-45)</b>	<b>45% (37-54)</b>	<b>39%(32-46)</b>

**Key:** N=Total sample number, ND=Not done (reach not sampled), NC = Not collected (reach was sampled, but fish were not collected in the defined time period).

<sup>1</sup> Note: All CWT includes 49 CWT Chinook salmon which had unreadable tags (no tag or unreadable tag code) or tag codes that could not be traced back to either hatchery. Therefore IGH and TRH CWT sample sizes are slightly smaller than the All CWT figures given.

## PARASITE PREVALENCE OF INFECTION IN NATURAL FISH AND SPORE DENSITY IN RIVER WATER AT THE KINSMAN SITE

Oregon State University conducts water sampling at numerous sites throughout the Klamath River (Hallett 2011, Hallett 2012). In 2012, additional water sampling was conducted at the Kinsman RST by Dr. Hallett (OSU). This site provides annual *C. shasta* POI data for natural Chinook salmon, as well as early infection status of IGH Chinook salmon following their release. Overlaying *C. shasta* actinospore density in water sampling with parasite POI in natural juvenile Chinook showed a trend of increasing infectivity by both measurements (Figure 3). *Ceratomyxa shasta* spore density in water samples (shown as gray X in Figure 3) corresponded well with increasing POI in natural fish tested (green triangles in Figure 3) from late April to late May at this site. It should be noted that spore densities in water at a particular point in time do not represent current parasite levels in fish. Natural fish likely had prior exposure at this site or other locations, prior to when water sampling occurred. However, water sampling conducted simultaneously with juvenile outmigration monitoring at a single site (concurrent weekly samples) can demonstrate increasing trends in infectivity in terms of both parasite exposure and developing infections in natural fish. Figure 5 also graphs *C. shasta* POI in mixed origin juvenile Chinook (blue circles) and CWT Chinook salmon (red triangles) but it must be noted that these were included only to provide temporal reference for the three types of fish groups tested. Mixed and CWT fish groups were collected at various sites as fish migrated downstream. The MOC and CWT POI data points were included in this graph to give the reader a relative sense of *C. shasta* POI over the entire sample period, compared to natural fish sampled at the Kinsman RST. Actinospore density data is for the Kinsman site only.



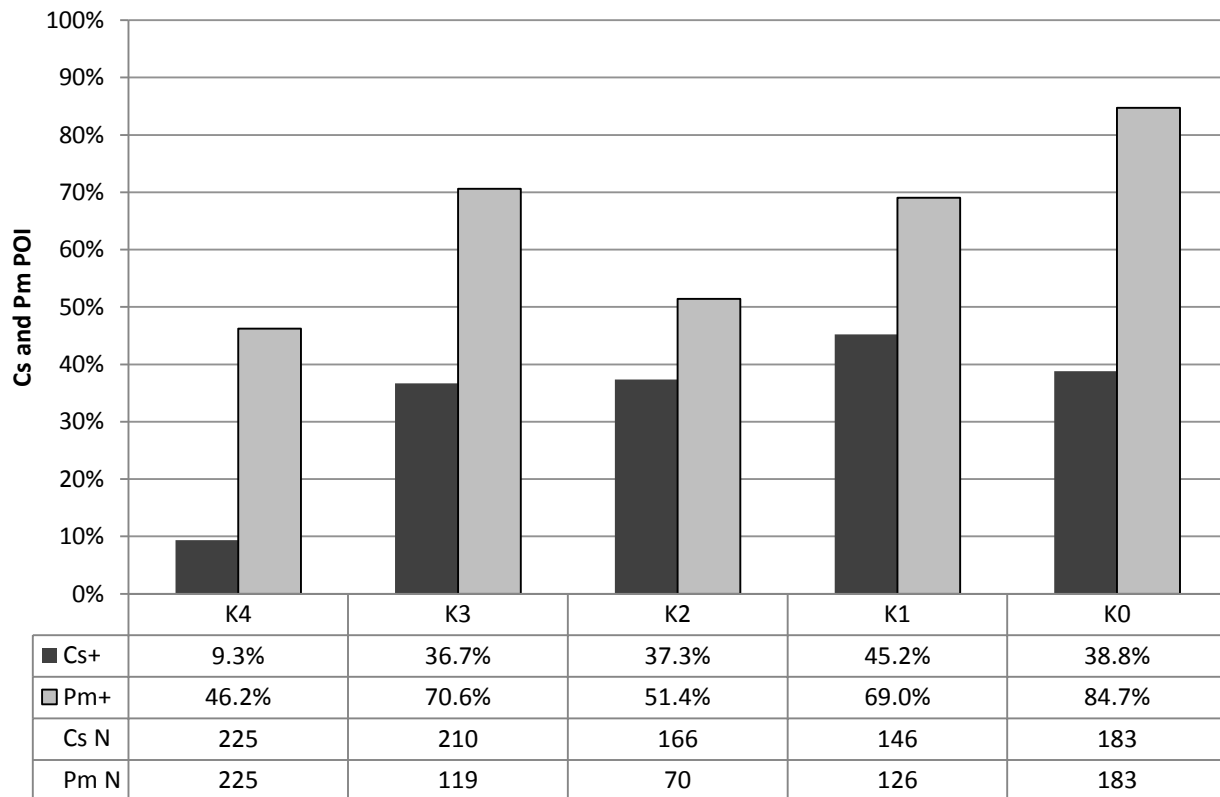
**Figure 3. Cycle Threshold (C<sub>T</sub>) values (y axis) denoting quantity of *C. shasta* DNA present in natural, mixed origin and coded-wire tagged (CWT) intestinal tissue of juvenile Chinook salmon. The infectious actinospore density (gray x) is also shown for environment water samples collected from the Kinsman rotary screw trap site (water testing and data provided by Dr. Sascha Hallett, Jerri Bartholomew laboratory, Oregon State University). For actinospore density data, a C<sub>T</sub> value of 33.4 correlates to 1 SPORE/L in environmental water samples.**

## PARASITE PREVALENCE OF INFECTION BY KLAMATH RIVER REACH

Prevalence of *C. shasta* infection in mixed origin Chinook salmon sampled throughout Klamath River was 31.9% (297/930, ci = 29-35%) similar to the annual metric of 30.4% (160/526, ci = 15-20%): the annual metric is restricted to above the Trinity confluence and May-July peak immigration period.

Prevalence was highest in the Trinity confluence to Estuary (K1) reach at 45.2%, followed by Estuary (K0) reach at 38.8%. Lowest prevalence was seen in K4 at 9.3% (Figure 4).

Prevalence of *P. minibicornis* infection in mixed origin Chinook salmon sampled in the Klamath River was 64.5% (466/723, ci = 86-91%). Prevalence was highest in K0 at 84.7%, followed by K3 at 70.6%. Lowest prevalence was seen in K4 at 46.2% (Figure 4).



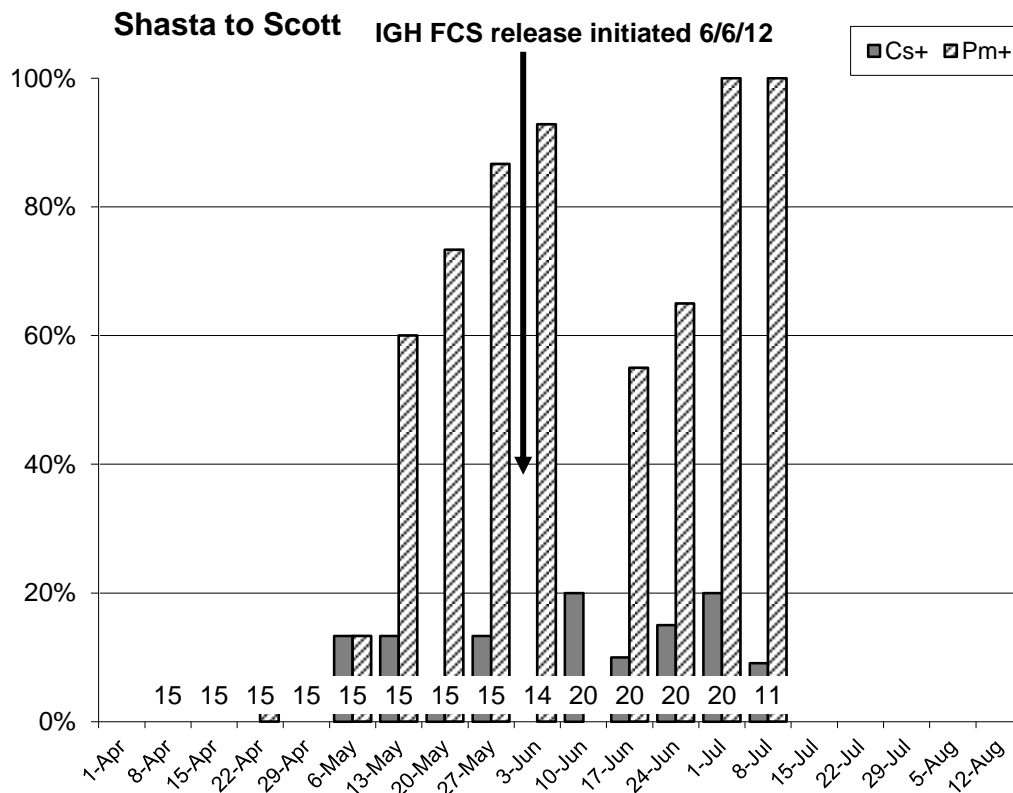
**Figure 4. Prevalence of *Ceratomyxa shasta* (Cs+) and *Parvicapsula minibicornis* (Pm+) infection in juvenile Klamath River Chinook salmon by capture reach. K4= Shasta to Scott; K3= Scott to Salmon; K2= Salmon to Trinity; K1= Trinity confluence to Estuary; K0= Klamath River Estuary. Sample numbers collected are displayed at the bottom of each column for both pathogens.**



### Shasta to Scott (K4) reach

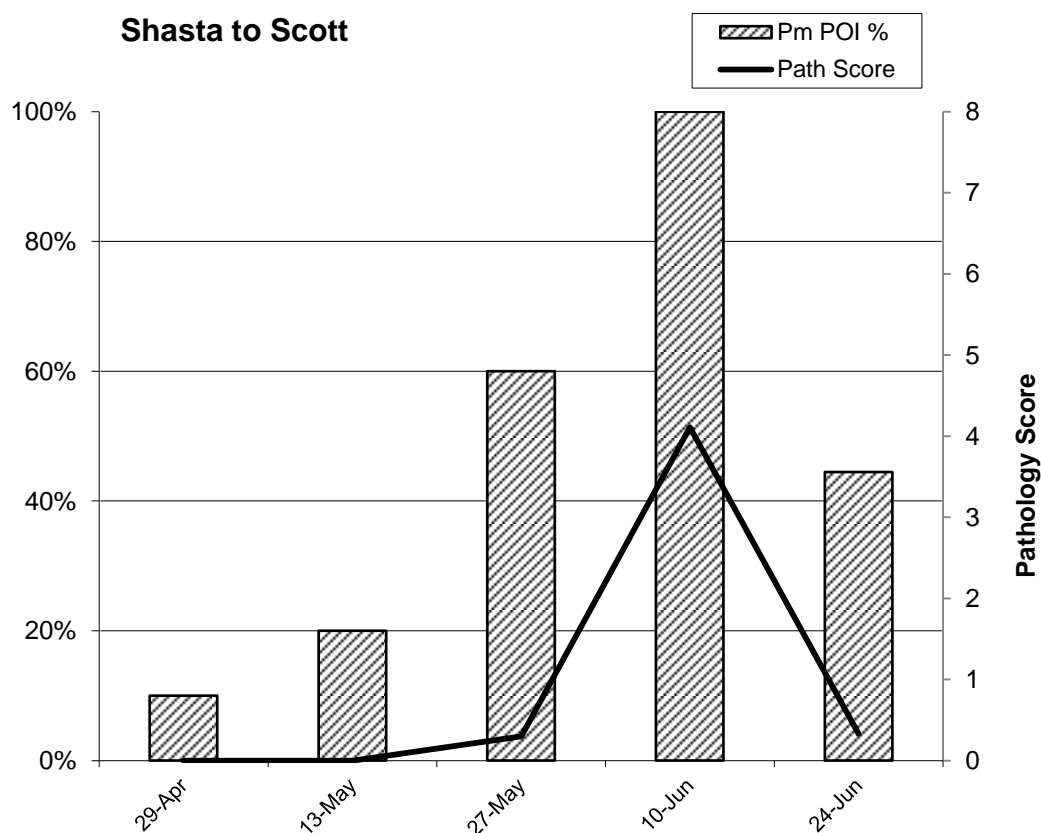
*Ceratomyxa shasta* was detected by QPCR in 9.3% (21/225, ci = 6-14%) of mixed-origin Chinook salmon which consisted primarily of natural fish. Generally, natural fish have lower *C. shasta* POI because these fish are captured when river temperatures are cooler (Apr-May). Prevalence peaked at 20% in early June and *C. shasta* was last detected in this reach at 9.1% in early-July. IGH initiated Chinook salmon release on June 6 (Figure 5). In the natural fish subset collected in this reach (April 11 to May 31), *C. shasta* was not detected in April, but was detected throughout most of May. *Ceratomyxa shasta* prevalence of infection in natural fish was 5.8% (7/120, ci = 2-12%). Once IGH fish are released, the POI in natural fish is masked by uninfected or low parasite prevalence in the newly exposed hatchery group.

*Parvicapsula minibicornis* was detected by QPCR in 46.2% (104/225, ci = 40-53%) of mixed-origin Chinook salmon (Figure 5). Infection prevalence reached 92.9% by early June, and decreased in mid to late June. Prevalence peaked at 100% at the end of July. *Parvicapsula minibicornis* POI in natural fish was lower at 30% (36/120, ci = 22-39%).



**Figure 5. Weekly prevalence of *Ceratomyxa shasta* (Cs+) and *Parvicapsula minibicornis* (Pm+) infection by QPCR in juvenile Klamath River Chinook salmon captured in K4 reach on the Klamath River (Shasta River to Scott River). Sample numbers collected and tested for *Ceratomyxa shasta* each week are displayed at the bottom of each column, while sub-sample numbers for *P. minibicornis* are listed in the sample table in Appendix C. *Parvicapsula minibicornis* was sampled on all dates except Apr 1: not detected on Apr 9, Apr 15, Apr 29, and Jun 10. *Ceratomyxa shasta* was not detected on Apr 8, Apr 15, Apr 22, Apr 29, Jun 3. Bold arrow indicates IGH FCS release date.**

Five bi-weekly histology collections occurred between April 29 and June 24 for a total of 50 specimens (Appendix A, Table A1). Collection groups between April 29 and May 27 were of natural origin while the June 10 and June 24 specimens were considered of mixed origin. *Ceratomyxa shasta* was detected in 8% of fish, and only on May 13, May 29, and June 10. Both natural and mixed origin salmon had low (10% and 5% respectively) prevalence of *C. shasta* infection. Individual trophozoites were found within granulomatous inflammation of the serosal surface of the intestine or associated visceral fat (had not invaded the intestine). In contrast, *P. minibicornis* infection of the kidney and metacercarial infection of the gill increased in the natural salmon beginning in mid-May. The kidney pathology score and gill lamellar hyperplasia also increased with time (Figure 6 and Appendix A1). Kidney pathology scores were considered low given the 6 – 8 range seen in clinically affected salmon in 2009. The influence of the IGH release on infection data was suggested by the decline in *P. minibicornis* prevalence and kidney pathology observed in June 24 sample (~ 3 weeks after first release). Another commonly observed abnormality was steatitis (inflammation of the visceral fat) in 45% of all fish examined (Appendix A, Table A1).

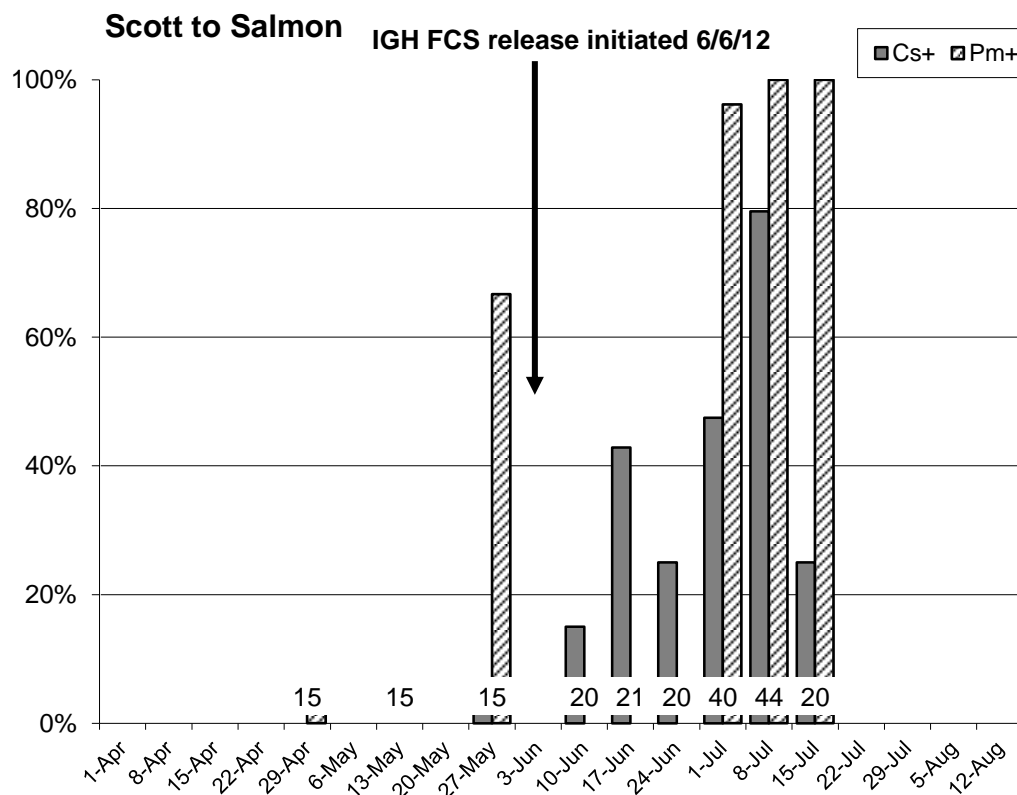


**Figure 6. Weekly prevalence of infection for *Parvicapsula minibicornis* (Pm POI %) and mean kidney pathology score (Path Score) by histology in juvenile Klamath River Chinook salmon captured in the Shasta to Scott (K4) reach.**

### Scott R. to Salmon R. reach (K3)

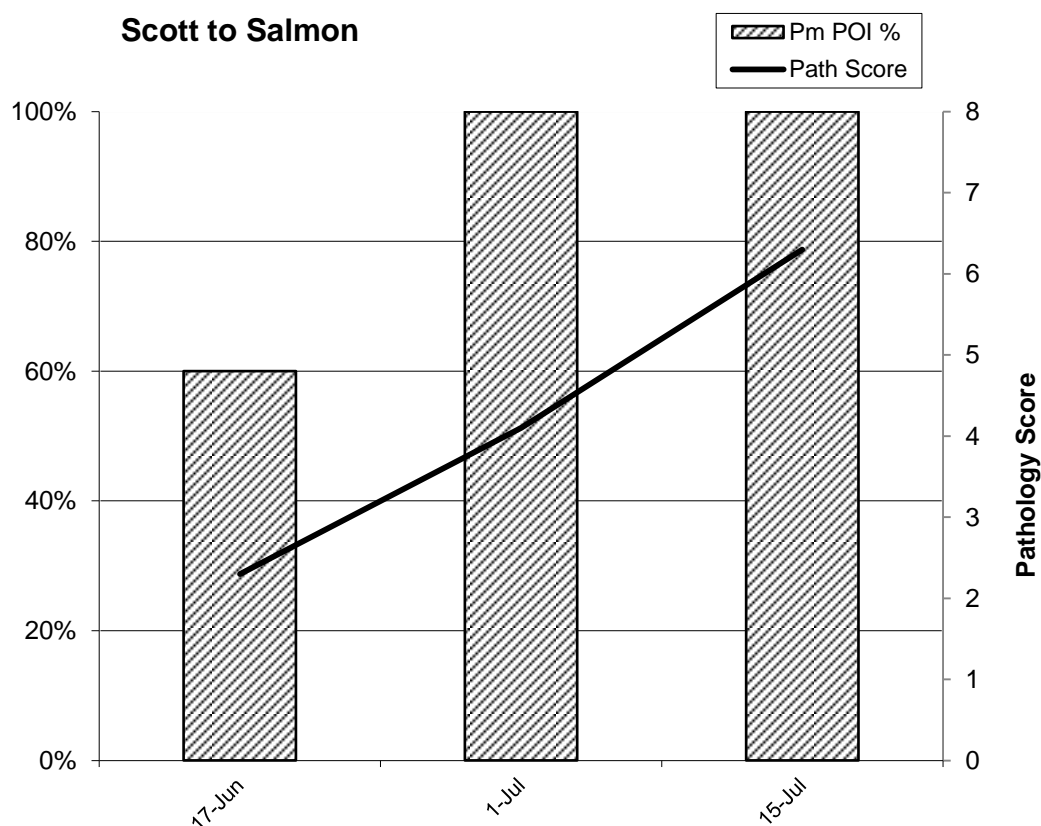
In the Scott to Salmon reach, *C. shasta* was detected by QPCR in 36.7% (77/210, ci = 30-44%) of mixed-origin Chinook salmon. Infection prevalence was first detected in late May at 6.7%, and ranged from 15-43% throughout June. The prevalence peaked at 79% in early July and dropped to 25% in the last sample of the season (Figure 7). Comparatively, *C. shasta* POI in natural fish (collected 30 Apr to 29 May) was 2.2% (1/45, ci = 0-3%) in this reach.

In this reach, *P. minibicornis* was detected by QPCR in 70.6% (84/119, ci = 62-79%) of mixed-origin Chinook salmon. Infection prevalence was first detected at 6.7% in late April, and increased to 66.7% by late May. The prevalence increased to 96.2% in early July and reached 100% one week later (Figure 7). *Parvicapsula minibicornis* POI in natural fish sampled in this reach was 24.4% (11/45, ci = 13-40%).



**Figure 7. Prevalence of *Ceratomyxa shasta* (Cs+) and *Parvicapsula minibicornis* (Pm+) infection by QPCR in juvenile Klamath River Chinook salmon captured in K3 reach on the Klamath River (Scott River to Salmon River). Sample numbers collected and tested for *Ceratomyxa shasta* each week are displayed at the bottom of each column, while subsample numbers for *Parvicapsula minibicornis* are listed in the sample table in Appendix C. *Parvicapsula minibicornis* was sampled on Apr 29, May 13, May 27, Jul 1, Jul 8, and Jul 15: not detected on May 13. *Ceratomyxa shasta* was not detected on Apr 29, and May 13.**

Three bi-weekly histology collections occurred between June 17 and July 15 for a total of 30 specimens (Appendix A, Tables A2). The June 17 collection was considered of natural origin while the July 1 and July 15 specimens were considered of mixed origin. Both natural and mixed origin salmon had low (10% and 5% respectively) prevalence of *C. shasta* infection. In contrast, *P. minibicornis* infection of the kidney and metacercarial infection of the gill was high ( $\geq 60\%$ ) in all collection groups. Kidney pathology scores increased over time to levels seen in clinically affected populations (True et al. 2010) (Figure 8). Inflammation of gill lamellae associated with metacercaria, presumptively *Apophallus* sp. (Ferguson et al. 2011), was seen in 90% of the fish. The metacercaria were associated with both epithelial hyperplasia and chondroid metaplasia.

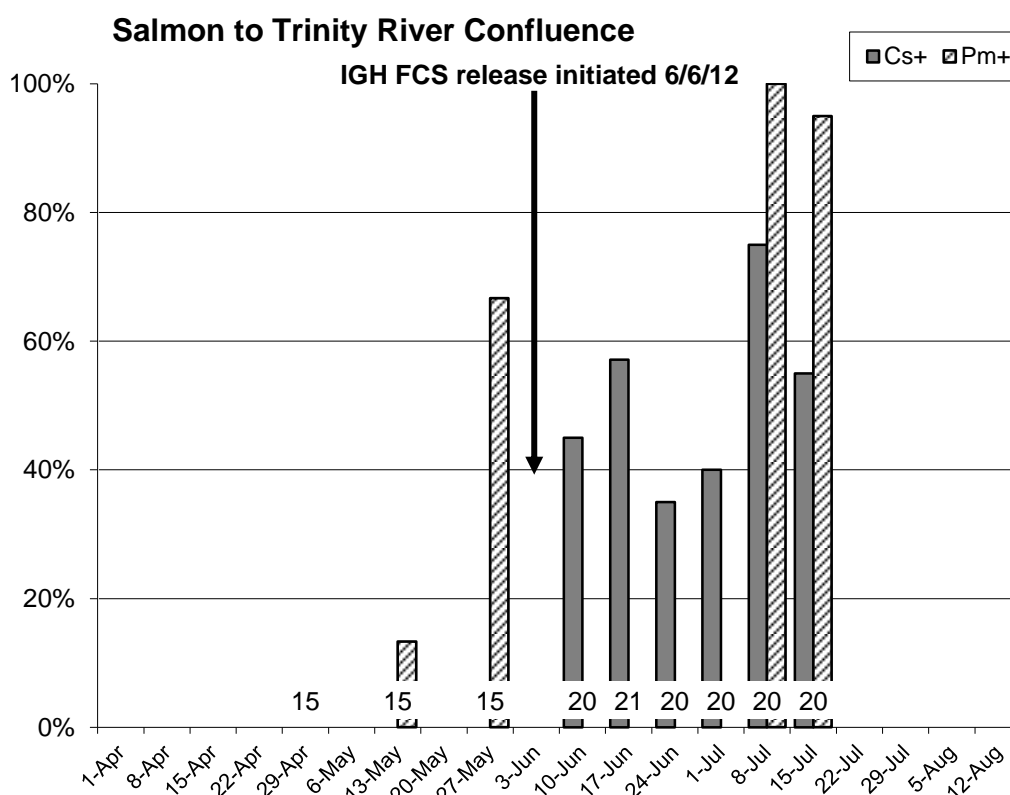


**Figure 8. Weekly prevalence of infection for *Parvicapsula minibicornis* (Pm POI %) and mean kidney pathology score (Path Score) by histology in juvenile Klamath River Chinook salmon captured in the Scott to Salmon (K3) reach.**

### Salmon R. to Trinity R. reach (K2)

In the Salmon to Trinity reach, *C. shasta* was detected by QPCR in 37.3% (62/166, ci = 30-45%) of mixed-origin Chinook salmon. The first detection of *C. shasta* occurred in early June at a prevalence of 45%. The prevalence increased to 57% on June 17 followed by two weeks with decreased levels. The prevalence peaked at 75% in early July (Figure 9). In contrast to the later mixed-origin group, *C. shasta* was not detected in natural Chinook salmon collected April 29 to May 27.

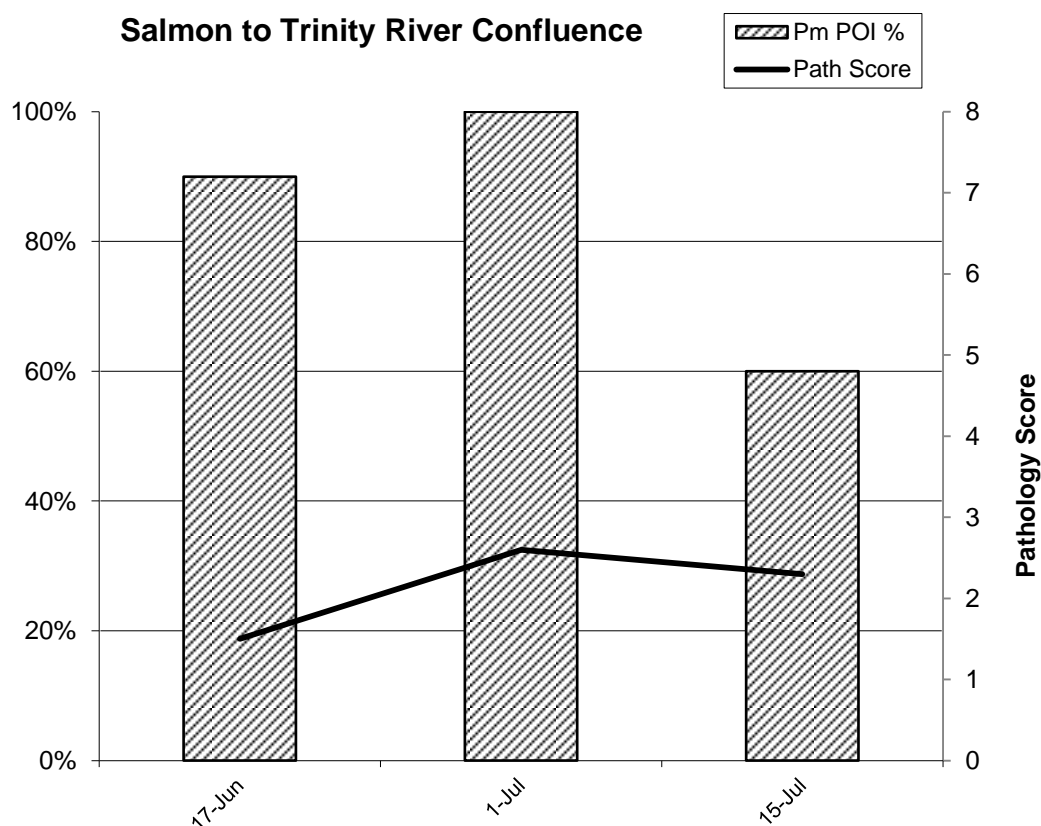
*Parvicapsula minibicornis* was detected by QPCR in 51.4% (36/70, ci = 39-64%) of mixed-origin Chinook salmon. Prevalence reached 66.7% by late May. In early July prevalence peaked at 100% (Figure 9). *Parvicapsula minibicornis* POI in natural fish, collected Apr 30 to May 29, was 26.7% (12/45, ci = 15-42%) in this reach.



**Figure 9. Prevalence of *Ceratomyxa shasta* (Cs+) and *Parvicapsula minibicornis* (Pm+) infection by QPCR in juvenile Klamath River Chinook salmon captured in K2 reach on the Klamath River (Salmon River to Trinity River). Sample numbers collected and tested for *Ceratomyxa shasta* each week are displayed at the bottom of each column, while subsample numbers for *Parvicapsula minibicornis* are listed in the sample table in Appendix C. *Parvicapsula minibicornis* was sampled on Apr 29, May 13, May 27, Jul 8, and Jul 15: not detected on Apr 29. *Ceratomyxa shasta* was not detected on Apr 29, May 13, and May 27.**



Three bi-weekly histology collections occurred between June 17 and July 15 for a total of 30 specimens (Appendix A, Table A3). The June 17 collection was considered of natural origin while the July 1 and July 15 specimens were considered of mixed origin. Mixed origin salmon had low (10%) prevalence of *C. shasta* infection. In contrast, *P. minibicornis* infection of the kidney and metacercarial infection of the gill was high ( $\geq 83\%$ ) in all collection groups. Kidney pathology scores were minor (Figure 10) while inflammation of gill lamellae associated with metacercaria, presumptively *Apophallus sp.* (Ferguson et al. 2011) was seen in all fish. The metacercaria were associated with both epithelial hyperplasia and chondroid metaplasia. One fish collected the week of July 15 had a single *Ichthyophthirius multifiliis* trophozoite in its gill section.

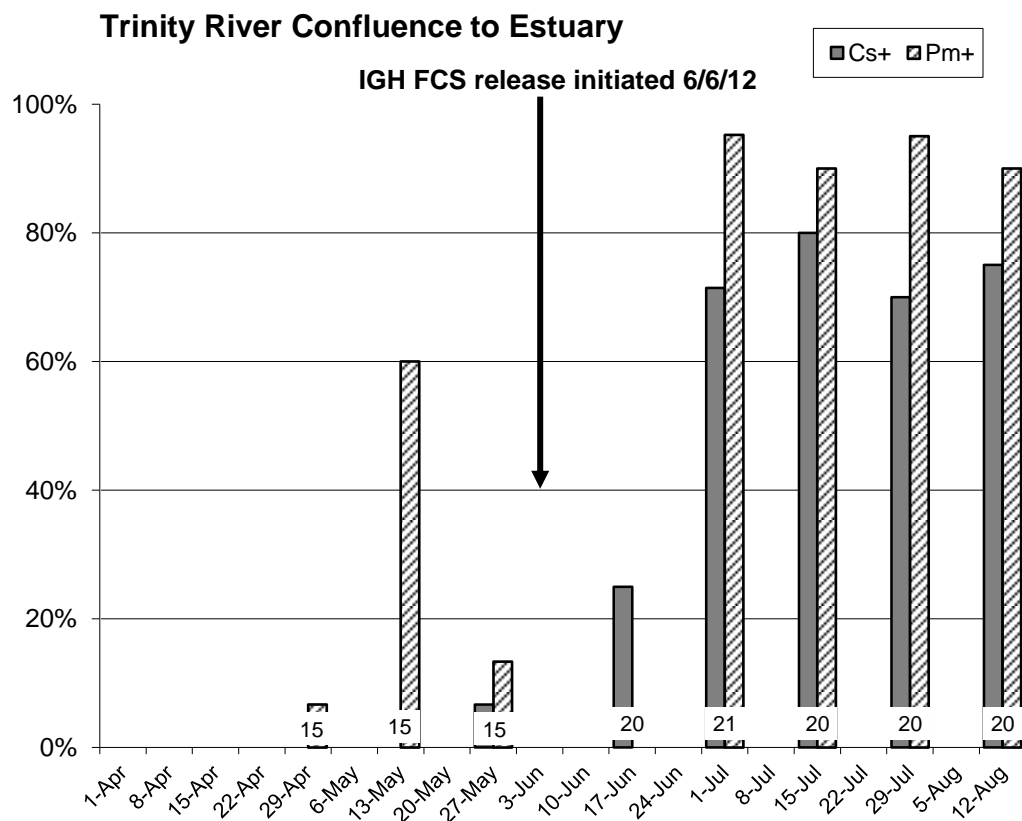


**Figure 10.** Weekly prevalence of infection for *Parvicapsula minibicornis* (Pm POI %) and mean kidney pathology score (Path Score) by histology in juvenile Klamath River Chinook salmon captured in the Salmon to Trinity (K2) reach.

### Trinity R. to Estuary reach (K1)

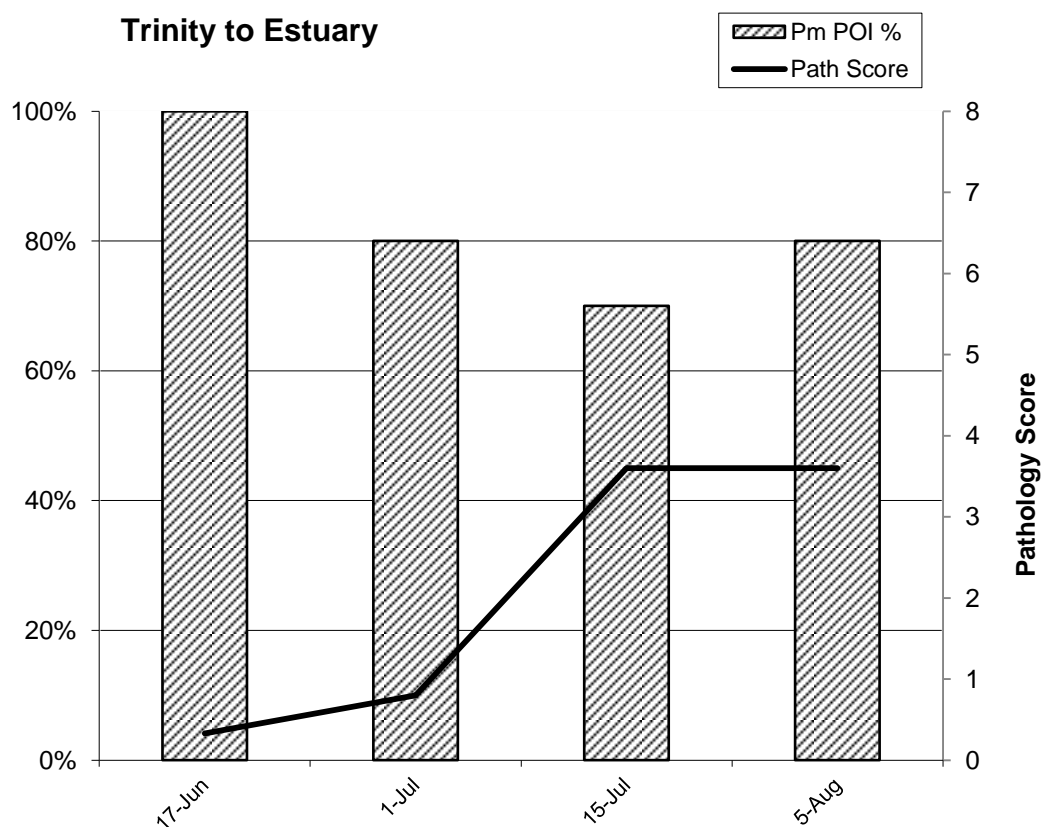
In the Trinity River to Estuary reach, *C. shasta* was detected by QPCR in 45.2% (66/146, ci = 37-54%) of mixed-origin Chinook salmon. Infection prevalence was expectedly low early in the season (Apr-May), peaked at 80% in mid-July, and then decreased slightly to 75% on the last sample date of 14 August (Figure 11). *Ceratomyxa shasta* POI in natural fish sampled in this reach (May 1 to May 29) was 2.2% (1/45, ci = 0-12%). Only five Chinook salmon of known Trinity origin (TRH-CWT) were captured in this reach; *C. shasta* was detected by QPCR in 40% (2/5, ci = 5-85%) and *P. minibicornis* was detected in 60% (3/5, ci = 15-95%).

In the Trinity River to Estuary reach, *P. minibicornis* was detected by QPCR in 69% (87/126, ci = 60-77%) of mixed-origin Chinook salmon. Infection prevalence peaked at 60% in natural fish sampled in mid-May, and was 26.7% (12/45, ci = 15-42%) for this group overall. *P. minibicornis* POI in mixed origin fish increased to 95.2% in early July. POI remained high for the remainder of the sampling period (90-95%, Figure 11).



**Figure 11 . Prevalence of *Ceratomyxa shasta* (Cs+) and *Parvicapsula minibicornis* (Pm+) infection by QPCR in juvenile Klamath River Chinook salmon captured in K1 reach on the Klamath River (Trinity River to the Estuary). Sample numbers collected and tested for *C. shasta* each week are displayed at the bottom of each column, while sub-sample numbers for *P. minibicornis* are listed in the sample table in Appendix C. *Parvicapsula minibicornis* was sampled on Apr 29, May 13, May 27, Jul 8, Jul 15, Jul 21 and Aug 12. *Ceratomyxa shasta* was not detected on Apr 29 and May 13.**

Four bi-weekly histology collections occurred between June 17 and August 5 for a total of 40 specimens (Appendix A, Table A4). The June 17 collection was considered of natural origin. Both natural and mixed origin salmon had low (10% and 3%) prevalence of *C. shasta* infection. In contrast, *P. minibicornis* infection of the kidney and metacercarial infection of the gill was high ( $\geq 60\%$ ) in all collection groups. Kidney pathology scores were generally low (Figure 12) however several individuals showed severe glomerulonephritis. Two gill sections had amoeba infections. Gill lamellae inflammation associated with metacercaria (presumptively *Apophallus* sp., Ferguson et. al.2011) was seen in 75% of the fish. The metacercaria were associated with both epithelial hyperplasia and chondroid metaplasia. One fish collected the week of August 5 had a single *Ichthyophthirius multifiliis* trophozoite in its gill section.



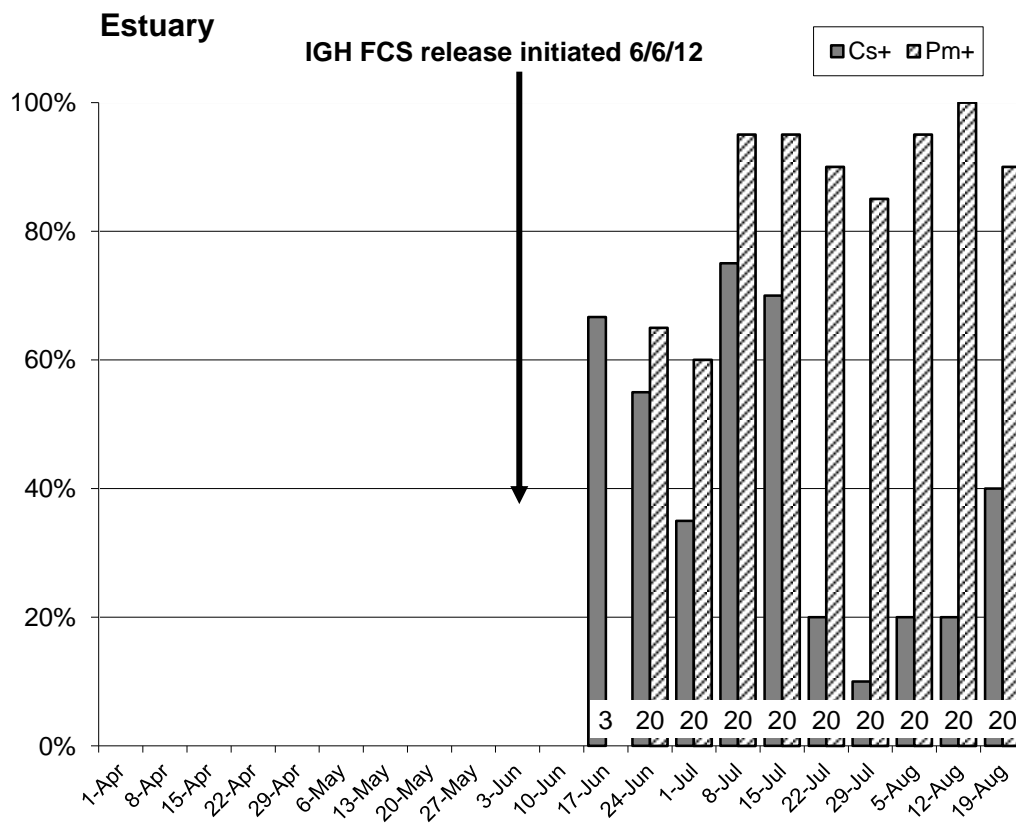
**Figure 12. Bi-weekly prevalence of infection for *Parvicapsula minibicornis* (Pm POI %) and mean kidney pathology score (Path Score) by histology in juvenile Klamath River Chinook salmon captured in the Trinity River confluence to Estuary (K1) reach.**

### Klamath River Estuary (K0)

In the Klamath River Estuary (K0) reach only CWT (IGH or TRH) Chinook were sampled. *Ceratomyxa shasta* was detected by QPCR in 38.8% (71/183, ci = 32-46%) of Chinook in this reach. Prevalence peaked in early July at 75% and then decreased and remained low (10-20%) through mid-August. *Ceratomyxa shasta* prevalence of infection increased again in the last sample collected 23 August to 40% (Figure 13).

*Parvicapsula minibicornis* was detected by QPCR in 84.7% (155/183, ci = 79-90%) of Chinook salmon collected in this reach. Infection was detected in late June at 65%, POI decreased one week later, but then increased to 95% in mid-Jul. Prevalence remained high (85-100%) for the remainder of the season.

Histology sampling was not performed in this reach.

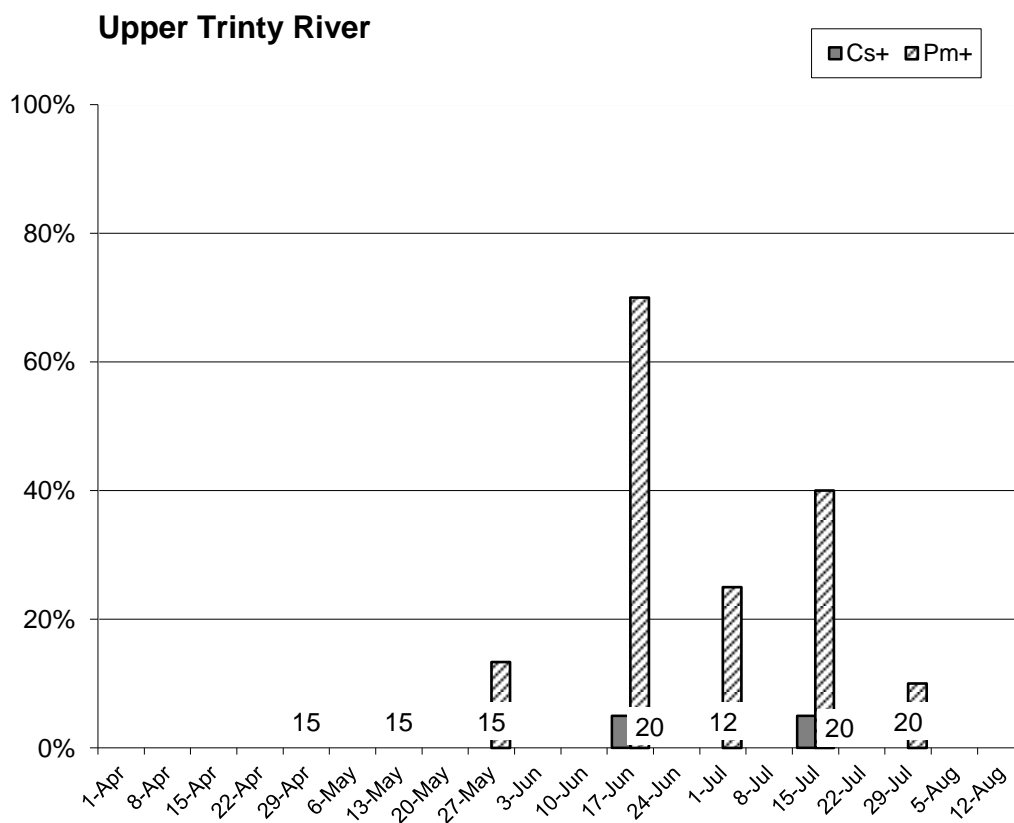


**Figure 13. Prevalence of *Ceratomyxa shasta* (Cs+) and *Parvicapsula minibicornis* (Pm+) infection by QPCR in juvenile Klamath River Chinook salmon captured in K0 reach on the Klamath River (Estuary). Sample numbers collected and tested for *C. shasta* and *P. minibicornis* each week are displayed at the bottom of each column. *P. minibicornis* was not detected on Jun 17.**

## PARASITE PREVALENCE OF INFECTION BY TRINITY RIVER REACH

### Upper Trinity River – Pear Tree Trap (T2)

In the upper Trinity River reach (T2), *C. shasta* was detected by QPCR in 1.7% (2/117, ci = 0-6%) of mixed-origin Chinook salmon. The two positive samples were CWT fish collected June 17 and July 15 (Figure 14). *Parvicapsula minibicornis* was detected by QPCR in 24.8% (29/117, ci = 17-34%) of mixed-origin Chinook salmon. *Parvicapsula minibicornis* POI was highest at 70% on June 17 (Figure 14). The majority of positive fish were CWT fish, with only 2 positive fish being of natural origin.



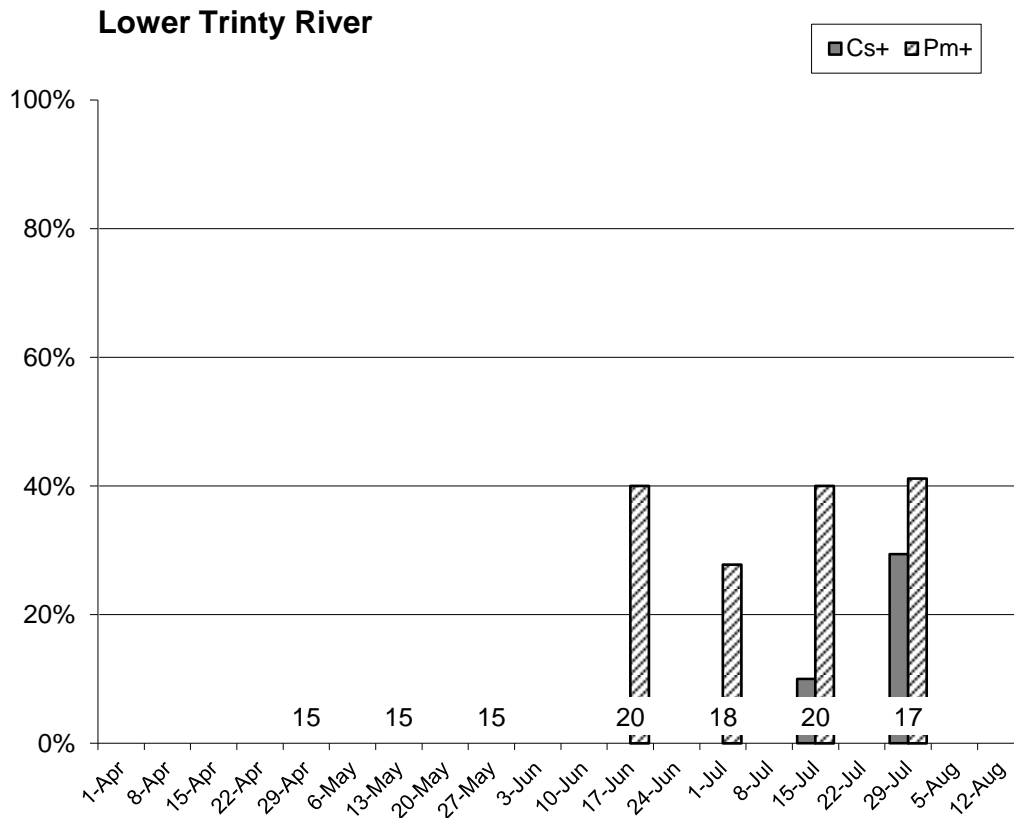
**Figure 14.** Prevalence of *Ceratomyxa shasta* (Cs+) and *Parvicapsula minibicornis* (Pm+) infection by QPCR in juvenile Trinity River Chinook salmon captured in T2 reach on the Trinity River (upper Trinity). Sample numbers collected and tested for *C. shasta* each week are displayed at the bottom of each column, while sub-sample numbers for *Parvicapsula minibicornis* are listed in the sample table in Appendix C. Sample collection started the week of Apr 29, but fish were not collected the week of Jun 10.

Two histology collections occurred in the weeks of May 27 and July 1 for a total of 19 specimens (Appendix A, Table A5). Neither *C. shasta* nor *P. minibicornis* were observed in the specimens. A kidney granuloma was observed in one fish collected in July and was not associated with either parasites or gram negative bacteria. It was characteristic of a bacterial kidney disease lesion. Common to Trinity R. salmon, the myxozoan kidney parasite *Chloromyxum* sp. and gill infections by mussel glochidia were detected in the fish. These parasites along with metacercaria cysts (presumptive *Nanophyetus salmonicola*) tend to be differentiating “biological tags” for Trinity R. juvenile Chinook. In contrast, Klamath R. juveniles tend to have metacercarial infections (presumptive *Apophallus* sp.) of the gill lamellae.



#### Lower Trinity River – Willow Creek Trap (T1)

*Ceratomyxa shasta* was detected in 5.8% (7/120, ci = 2-12%) of the mixed origin Chinook salmon screened by QPCR (Figure 15). All positive fish were CWT fish and were collected on either July 16 or August 8. *Parvicapsula minibicornis* was detected in 23.3% (28/120, ci = 16-32%) of mixed origin Chinook salmon screened by QPCR (Figure 15). Similar to *C. shasta*, the majority of positive fish were CWT juvenile Chinook collected from the lower Trinity River site.



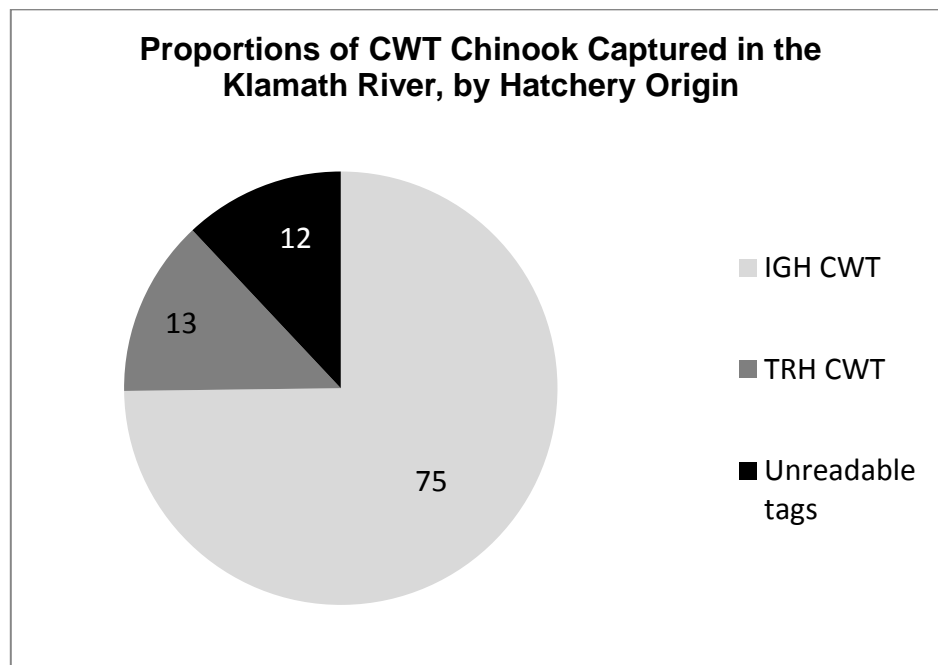
**Figure 15. Prevalence of *Ceratomyxa shasta* (Cs+) and *Parvicapsula minibicornis* (Pm+) infection by QPCR in juvenile Trinity River Chinook salmon captured in T1 reach on the Trinity River (lower Trinity). Sample numbers collected and tested for *C. shasta* each week are displayed at the bottom of each column, while sub-sample numbers for *Parvicapsula minibicornis* are listed in the sample table in Appendix C. Sample collection started the week of Apr 29, but fish were not collected the week of Jun 10.**

Two histology collections occurred in the weeks of May 27 and July 1 for a total of 17 specimens (Appendix A, Table A6). *Parvicapsula minibicornis* was not observed in the specimens. Common to Trinity R. salmon, the myxozoan kidney parasite *Chloromyxum sp.* and gill infections by mussel glochidia were detected in the fish. Two gill sections contain presumptive *C. shasta* trophozoites however the parasite was not seen in the intestine sections.

In summary, the overall prevalence of *C. shasta* infection in mixed origin Chinook salmon sampled from both Trinity River locations was 3.8% (9/237, ci = 2-7%). Prevalence was higher (5.8%) in the lower Willow Creek RST (T2 site) compared to the upper Pear Tree RST (T1 site) at 1.7%. For *P. minibicornis* infection in mixed origin Chinook salmon sampled in the Trinity River the prevalence of infection was slightly higher at the upper site (24.8%) but similar to the lower Willow Creek site (23.3%).

### PARASITE PREVALENCE OF INFECTION IN MARKED (CWT) CHINOOK SALMON

The 25% constant fractional mark rate at Iron Gate Hatchery (Buttars and Knechtle, 2009) has permitted the capture of a large proportion of IGH CWT Chinook salmon in the past four years of the monitoring program. A total of 409 CWT Chinook salmon were collected this season from the upper and lower reaches of the Klamath River with IGH CWT fish accounting for 74.8% (306/409) and TRH CWT fish accounting for 13.2% (54/409) of coded wire tagged fish tested. Forty nine coded wire tags (12%) recovered from the Klamath River were unreadable, or could not be identified to hatchery releases (Figure 16). An additional 145 TRH CWT fish were collected in the Trinity River, primarily at the Willow Creek trap. In addition to prevalence of infection data reported previously for CWT Chinook (page 10), these tagged Chinook provide a method of assessing temporal myxozoan infection level at weeks post hatchery release.

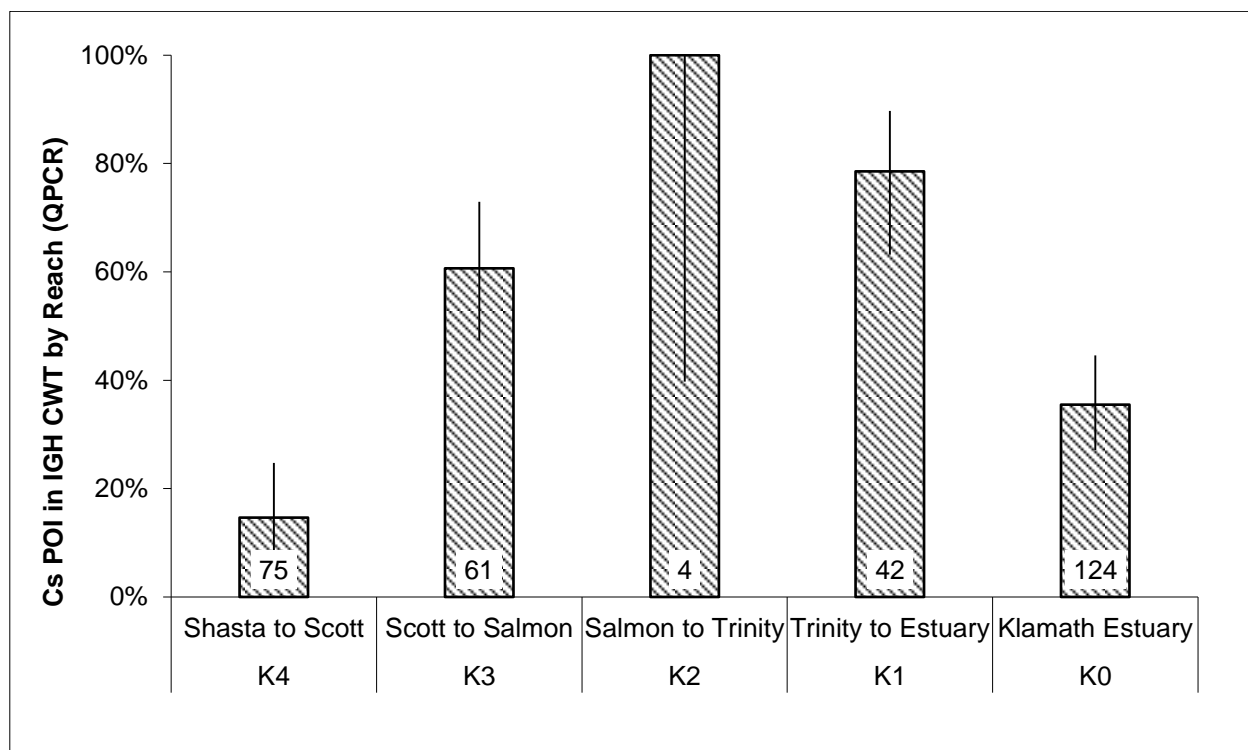


**Figure 16. Proportions of CWT Chinook salmon originating from IG or TR Hatcheries. Unreadable tags: 49 CWT Chinook salmon which had unreadable tags (no tag or unreadable tag code) or tag codes that could not be traced back to either hatchery.**

### Iron Gate Hatchery

Coded wire tagged salmon originating from IGH were collected from various reaches of the Klamath River from June 13 to August 23. The largest proportions of IGH CWT Chinook salmon were recovered from the Estuary (K0) and the Shasta River to Scott River (K4) reaches.

*Ceratomyxa shasta* was detected in 42% (129/306, ci = 37-48%) of all IGH CWT Chinook salmon screened by QPCR and 37% (52/140, ci = 29-36%) of IGH CWT collected above the confluence of the Trinity River (Figure 17). *Ceratomyxa shasta* POI was low (15%) in the upper reach (K4) and highest in Chinook salmon recovered from the Salmon River to Trinity River reach (K2) at 100% (however only 4 CWT fish were sampled in this reach). *Ceratomyxa shasta* POI in IGH CWT collected from the Trinity River to the Estuary (K1) reach was 79% (33/42, ci = 63-90%), and 35% in Estuary (K0).



**Figure 17. *Ceratomyxa shasta* prevalence of infection (POI) by QPCR in Iron Gate Hatchery CWT by reach in which marked Chinook salmon were recovered from. Whiskers indicate 95% confidence interval; sample numbers collected and tested for *C. shasta* each week are displayed at the base of each column.**

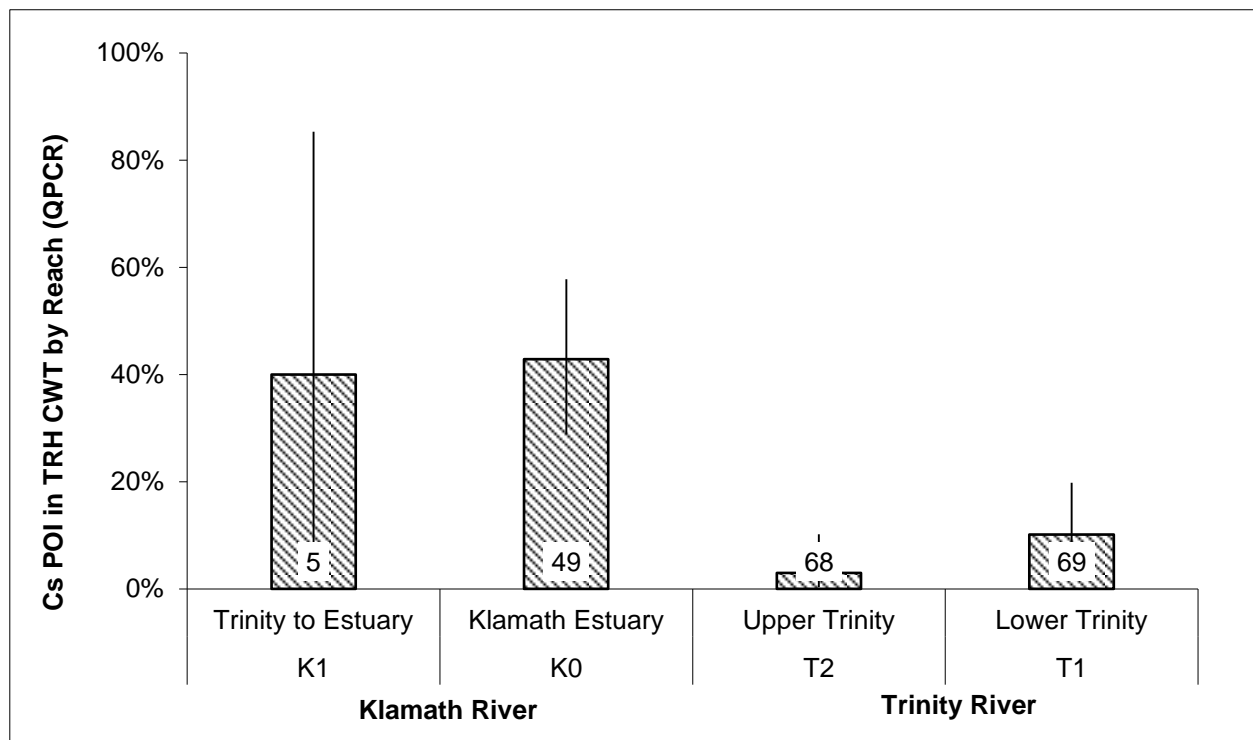
*Parvicapsula minibicornis* was detected in 89% (272/306, ci = 85-92%) of all marked IGH Chinook salmon screened by QPCR and 81% (113/140, ci = 73-87%) of IGH-CWT collected above the confluence of the Trinity River.

### Trinity River Hatchery

Coded wire tagged salmon originating from TRH were collected from June 20 through August 3 at two Trinity River sites, and June 20 to August 23 in the lower Klamath River. The largest proportion of Trinity River Hatchery CWT Chinook salmon were recovered from the Willow Creek rotary screw trap in the lower Trinity River reach (T1), accounting for 36.1% (69/191) of all TRH CWT tested.

In marked TRH Chinook, *C. shasta* was detected in 16.8% (32/191, ci = 12-23%) of all salmon screened by QPCR. *Ceratomyxa shasta* POI was higher in Chinook salmon recovered from the Trinity to Estuary reach (K1) at 40% (2/5, ci = 5-85%) and the Estuary reach (K0) at 42.9% (21/49, ci = 29-58%) (Figure 18), compared to fish collected in the Trinity River at 6.6% (9/137, ci = 3-12%).

*Parvicapsula minibicornis* was detected in 43.5% (83/191, ci = 36-51%) of marked TRH Chinook salmon screened by QPCR.



**Figure 18.** *Ceratomyxa shasta* prevalence of infection (POI) by QPCR in Trinity River Hatchery CWT by reach in which marked Chinook salmon were recovered from. Whiskers indicate 95% confidence intervals. Sample numbers collected and tested for *C. shasta* CWT are displayed at the base of each column.

### Prevalence of Infection and Parasite Load at Weeks At Large

Historical *C. shasta* prevalence of infection date for IGH and TRH CWT detected by QPCR and histology are given in Table 5. The methodology used for reporting the annual disease metric has changed over the seven years of the monitoring program. Prevalence of infection by histology had been utilized as the metric for annual comparisons of disease prevalence (data confined to peak migration period of May-July and above the Trinity confluence) from 2006-2008. The annual metric transitioned to QPCR data in 2009, with the same data restrictions, due to the higher sensitivity of this method in detecting early and/or low-level parasite infections and the ability to quantify parasite DNA copy number within fish tissue.

The benefit of concurrent testing with QPCR is the ability to provide quantitative assessments of parasite load within migrating juvenile Chinook salmon collected within various reaches and eventually the Estuary. Quantitative parasite load permits assessment of the relationship between WAL and infection intensity in CWT Chinook salmon (see discussion). Supplemental histology continues to be performed annually for select reaches to assess tissue damage associated with clinical disease and to detect other pathogens that may be present. Histology is not performed on CWT Chinook salmon but is performed at similar sample sites and periods, utilizing mixed-origin fish groups.

**Table 5. Historic annual prevalence of *Ceratomyxa shasta* infection (% positive by assay) in all juvenile Chinook salmon collected from the Klamath main stem between Iron Gate Dam and Trinity River confluence during May through July, 1995-2012. Similar data is shown in columns 4 for IGH CWT captured in reaches above the confluence of the Trinity River (K5, K4 and K2) and for TRH CWT collected below the Trinity R. confluence (K1) and estuary (K0).**

Year	Annual <i>C. shasta</i> POI <sup>1</sup> All Chinook (May 1 – July 30)		Iron Gate CWT (% Positive - QPCR)	Trinity CWT (% Positive - QPCR)
	(% Positive by Assay) Histology	QPCR		
2006	21	34	6/18 (33%)	1/67 (1%)
2007	21	31	15/22 (68%)	46/332 (14%)
2008	37	49	9/13 (69%)	8/257 (3%)
2009	54	45	82/228 (36%)	13/100 (13%)
2010	15	17	17/149 (11%)	1/45 (2%)
2011	2 <sup>2</sup>	17	22/176 (13%)	4/49(8%)
2012	9	30	52/140 (37%)	19/44 (43%)
Average (SE)	23% (7)	32% (5)	27% (9)	10% (6)

<sup>1</sup> K5 reach (IG dam to the Shasta) was sampled each year, prior to 2011. *C. shasta* POI has historically been very low in this reach.

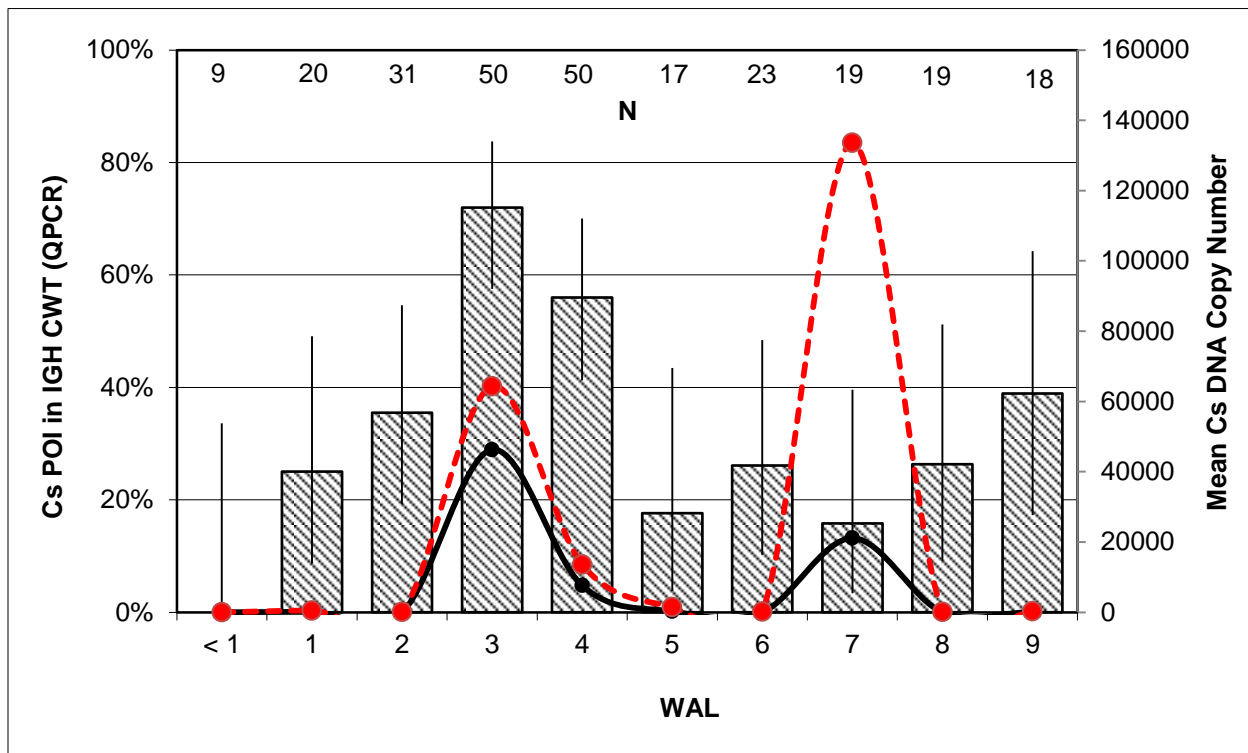
<sup>2</sup> Histology sampling was reduced and limited to 2 reaches in 2011: Shasta to Scott (K4) and Trinity confluence to Estuary (K1).



### Iron Gate Hatchery

*Ceratomyxa shasta* parasite load, as determined by quantity of *C. shasta* DNA detected in the intestine of Chinook salmon, is a measure of infectious load at the time fish are captured. The parasite load in CWT Chinook salmon, given in DNA copy number, was bi-modal in 2012 (Figure 18), similar to trends observed in previously monitoring years (Bolick et al. 2011, True et al. 2010). This bi-modal distribution is likely associated with physiological smolt status which drives rate of migration in juvenile Chinook salmon following release from IGH. The majority of fish likely are actively migrating while smaller/younger fish may reside for a few weeks before continuing to the Estuary.

*Ceratomyxa shasta* DNA copy number was highest in IGH CWT Chinook salmon residing 7 WAL, followed by fish residing 3 WAL. The average *C. shasta* parasite copy number for infected fish residing 3 WAL was ~60,000 copies and two times higher (~130,000 copies) in the 7 WAL group (Figure 19). However, *C. shasta* POI was highest in the 3 WAL group (>70%), and less than 20% in the 7 WAL group. This indicates that while a larger proportion of the Chinook salmon residing 3 WAL upon recapture were infected, the parasite infectious load was lower than that observed in fish residing 7 WAL. The 7 WAL group had fewer infected fish, but the fish that were infected had high parasite loads. In the 5 WAL group, *C. shasta* prevalence of infection and DNA copy number are well correlated. As fish become clinical, moribund fish likely dropped out of the population, which resulted in a decrease in *C. shasta* POI. Note the POI level is nearly inverse in the later groups (lower POI associated with highest DNA copy number).



**Figure 19.** *Ceratomyxa shasta* prevalence of infection in IGH CWT by Weeks At Large (WAL) post hatchery release. Lines (dashed red) are the mean *C. shasta* DNA copy number for Chinook salmon testing positive by QPCR, and (solid black) mean DNA copy number for all Chinook salmon tested by QPCR.

Compared to *C. shasta*, *P. minibicornis* prevalence of infection in IGH CWT, and historically in the entire mixed origin population, is generally much higher throughout the late spring and summer sampling period. In terms of WAL data, *P. minibicornis* infectious load was highest for the 3-4 WAL groups consisting of parasite DNA copy numbers above 190,000 copies (Figure 20). Prevalence of infection remained high (88-100%) in the 2-9 WAL groups. However the parasite infectious load decreased after 4 WAL and remained relatively low with the exception of a second smaller peak in the 6 WAL group.

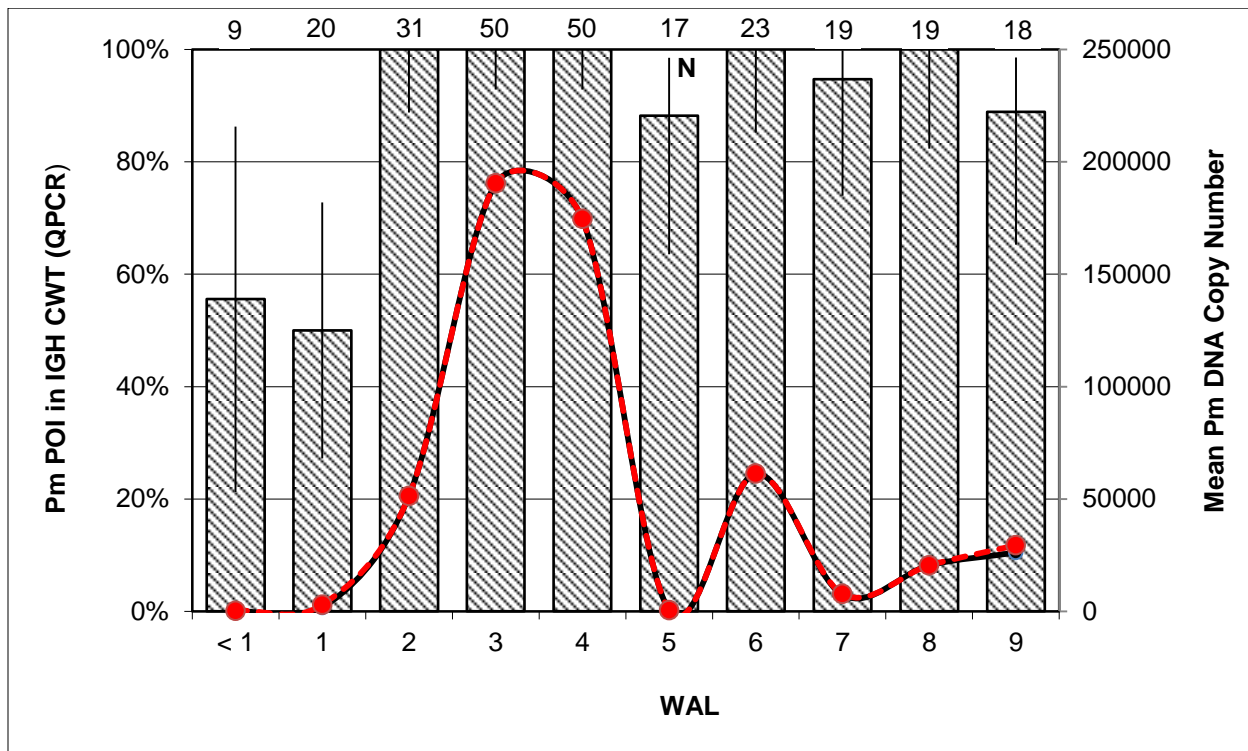
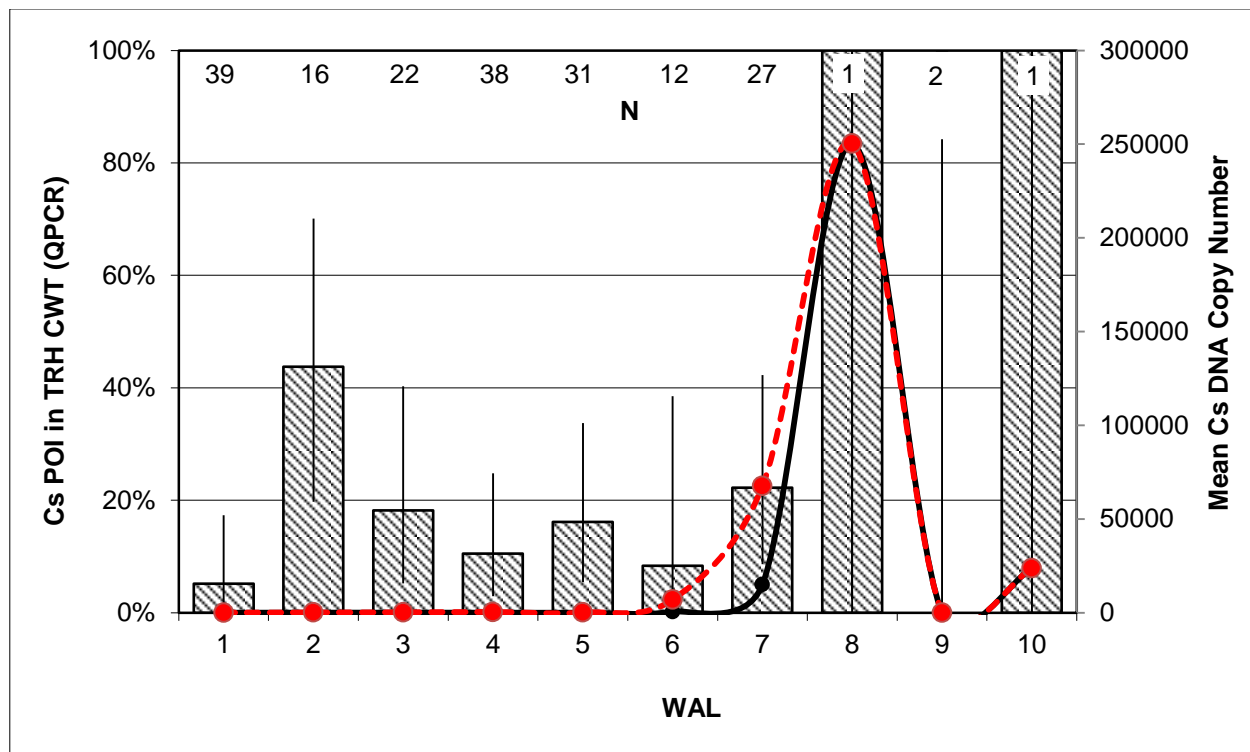


Figure 20. *Parvicapsula minibicornis* prevalence of infection in IGH CWT by Weeks At Large (WAL) post hatchery release. Lines (dashed red) are the mean *P. minibicornis* DNA copy number for Chinook salmon testing positive by QPCR, and (solid black) mean DNA copy number for all Chinook salmon tested by QPCR.

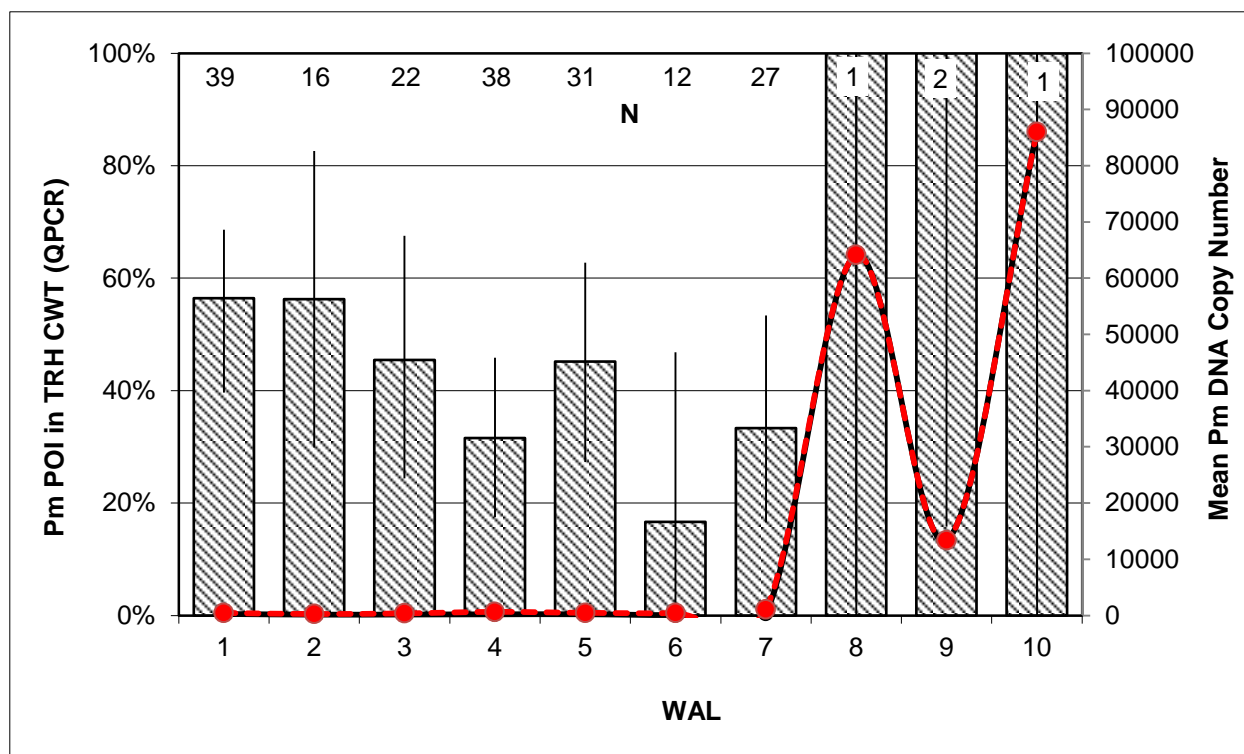
### Trinity River Hatchery

*Ceratomyxa shasta* parasite load, as determined by parasite DNA copy number, was highest in TRH CWT Chinook salmon residing for 7-10 WAL, however sample size was extremely small for the 8-10 WAL groups (Figure 21). Two *C. shasta* positive fish residing 7 and 8 WAL had clinical disease (hemorrhagic intestine) upon dissection and DNA copy number of approximately 450,000 and 250,000 respectively. The majority of the remaining *C. shasta* positive TRH CWT Chinook had low DNA copy numbers ranging from 20 to 650, with the exception of one fish at 7000 copies.



**Figure 21.** *Ceratomyxa shasta* prevalence of infection in TRH CWT by Weeks At Large (WAL) post hatchery release. Lines (dashed red) are the mean *C. shasta* DNA copy number for Chinook salmon testing positive by QPCR, and (solid black) mean DNA copy number for all Chinook salmon tested by QPCR.

*Parvicapsula minibicornis* parasite load was highest in TRH CWT Chinook salmon residing 8-10 WAL, but again only 4 fish were represented in these groups (Figure 22). Trinity River Hatchery CWT Chinook salmon had negligible levels of *P. minibicornis* DNA present in the kidney, and prevalence of infection ranged from 18-48% in 1-6 WAL groups. Similar to *C. shasta*, *P. minibicornis* parasite load is highest (~15,000-90,000 copies) in the few fish in the 8-10 WAL group, but this is approximately half the level observed in IGH CWT Chinook salmon. These four *P. minibicornis* positive TRH CWT fish were recaptured in the Estuary.



**Figure 22.** *Parvicapsula minibicornis* prevalence of infection in TRH CWT by Weeks At Large (WAL) post hatchery release. Lines (dashed red) are the mean *P. minibicornis* DNA copy number for Chinook salmon testing positive by QPCR, and (solid black) mean DNA copy number for all Chinook salmon tested by QPCR.

## Environmental Conditions

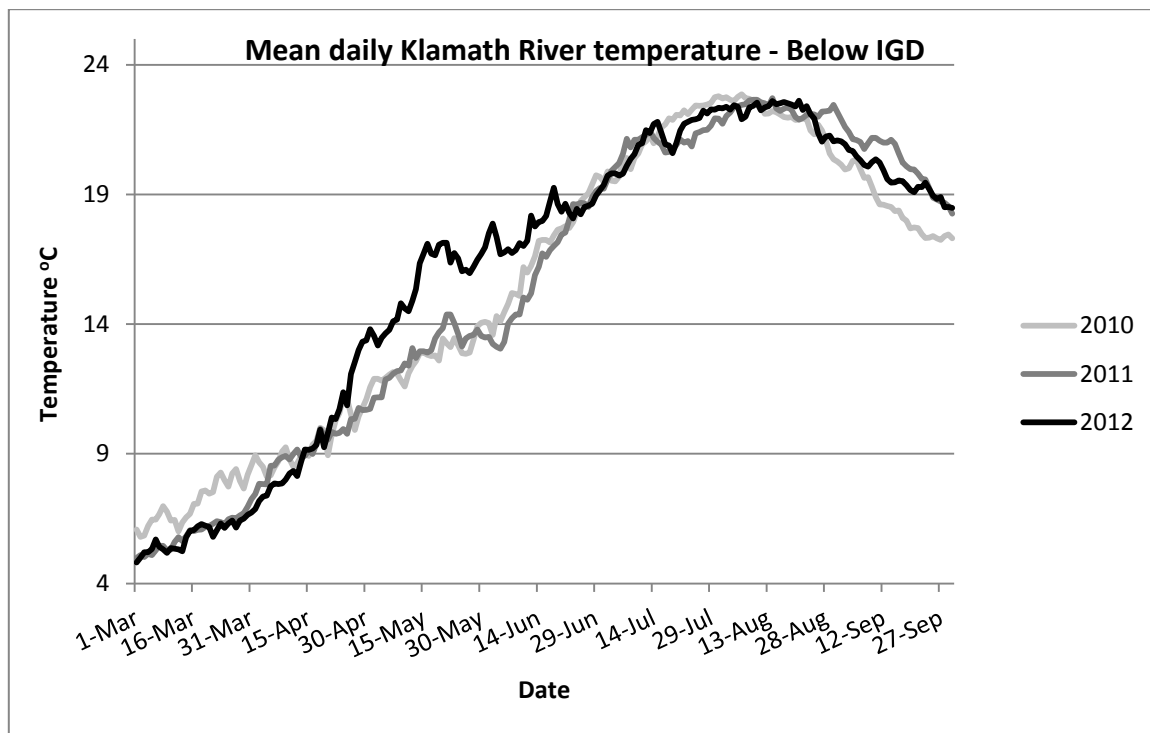
### Temperature

In previous study years (2006-2009) we typically observed water temperatures of approximately 18 °C (and often as high as 22 °C) in mid to late May and steadily increasing during the peak juvenile migration period of May through July. However in 2010 and 2011, average river temperatures were cooler in spring and for an extended period in May and June (Figure 23). These cooler temperatures resulted in lower *C. shasta* POI (17% by QPCR for both years), and the lowest levels observed to date. Historically *C. shasta* POI ranged from 31-49% during 2006-2009 study years (Table 5).

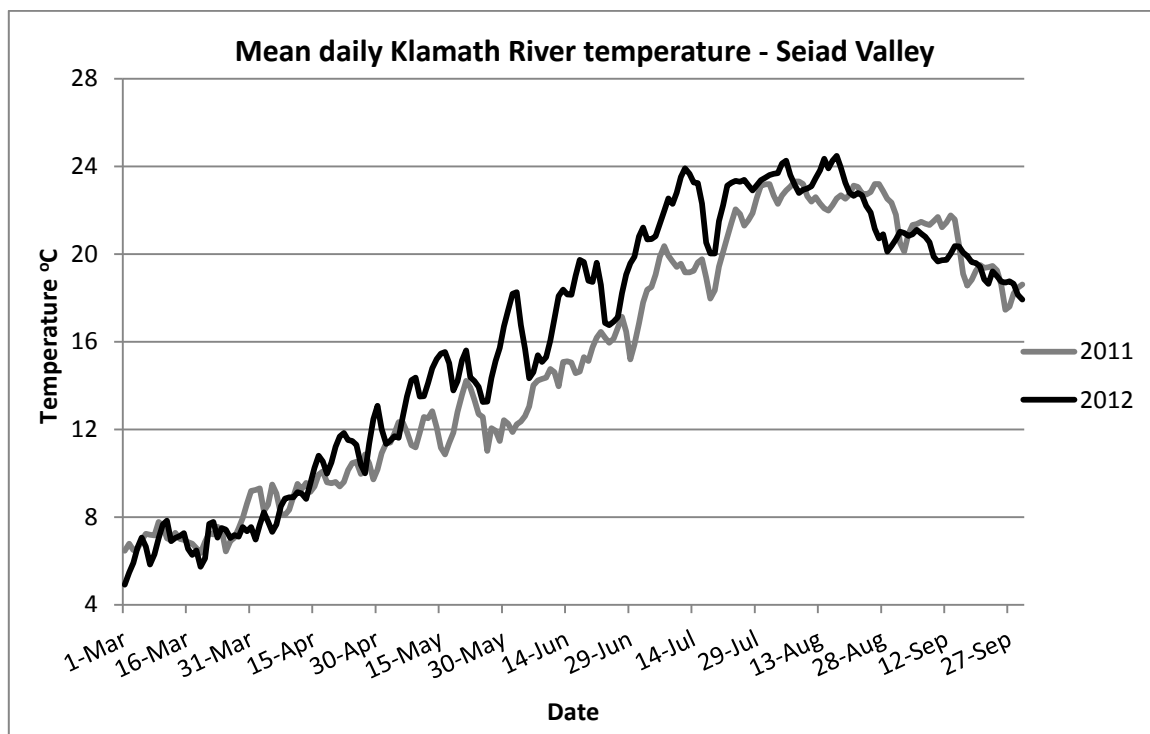
The river temperatures observed in 2012 appeared to be more typical and similar to historic temperatures, in terms of mean daily river temperature below IGD. In 2012, temperatures began diverging from those observed in 2010-2011 on April 20, reached 18 °C by mid to late May and then were consistently above 18 °C by mid-June (Figure 23). Mean daily temperature appears to be 3-4 degrees warmer from late April to mid-June in 2012 compared to 2010-2011. Mean daily temperature trajectory and magnitude appears similar from late June forward for all three years shown (Figure 23).

At the Seiad Valley temperature gauge, mean daily river temperatures were more sporadic but higher than 2010-2011 from approximately early May to mid-July (with the exception of a decrease in temperature in late June) (Figure 24). Average daily temperatures reached 18 °C on 30 May for a brief period of time, and then consistently reached this temperature from mid-June forward. At several points

(late May, mid-June and mid-July), the difference in temperature in this part of the river was ~ 4-5 degrees warmer than observed in 2010-2011.



**Figure 23.** Mean daily temperature below Iron Gate Dam for 2010, 2011, and 2012. Temperature data for 2010 and 2011 acquired from Arcata Fish and Wildlife Field Office. Temperature data for 2012 acquired from Iron Gate Hatchery, measurements taken from the mainstem Klamath River, not the hatchery facility.



**Figure 24.** Mean daily temperature from March through September 2011 and 2012 at Seiad Valley. Temperature data acquired from Arcata Fish and Wildlife Field Office.

### River Flows

In 2012, Klamath River flows below Iron Gate Dam increased gradually in March and remained between 3000-4000 cfs from the period of early April to mid-May. Flows declined to 2000 cfs by early June, and were approximately 1600 cfs throughout June (Figure 25). IGH fish were released June 6-18.

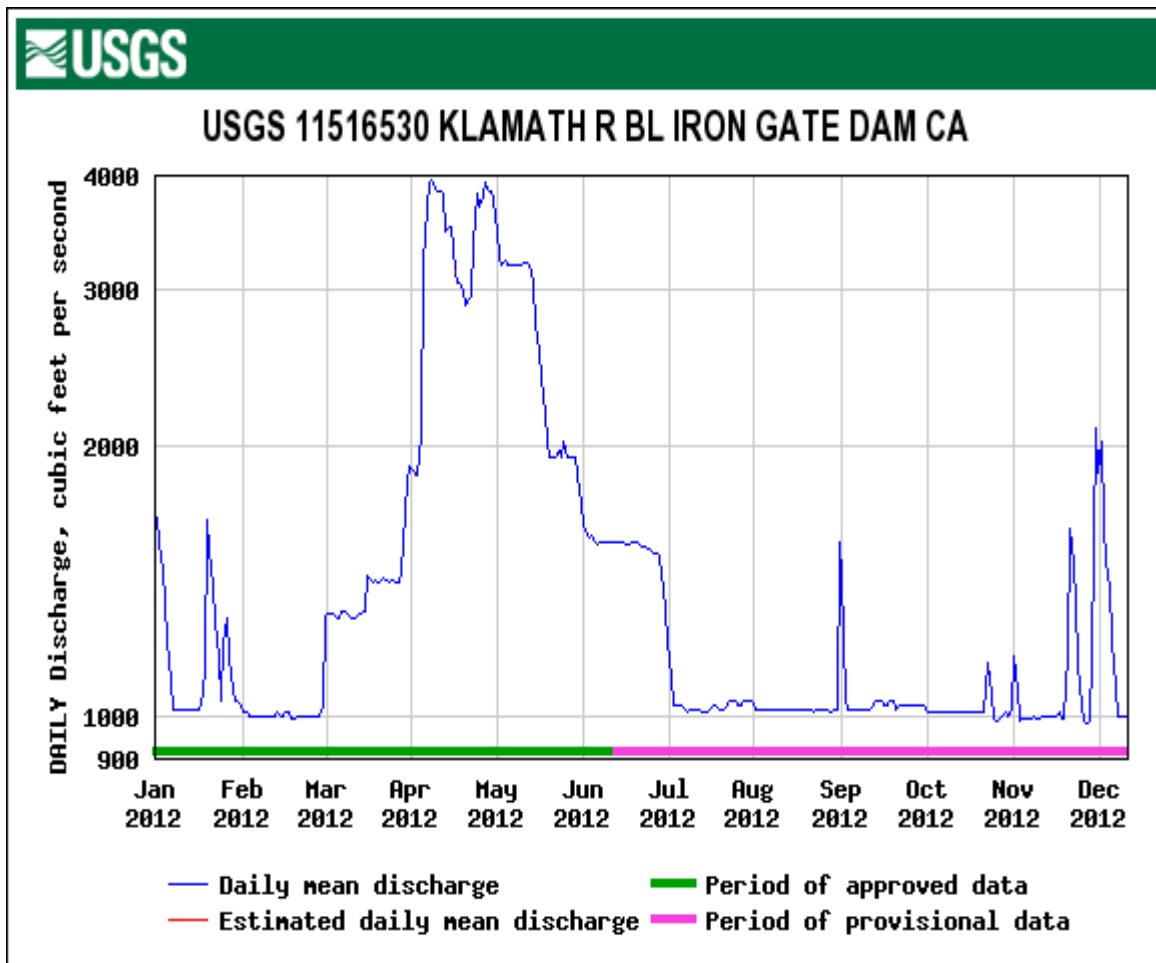


Figure 25. Daily discharge (log cfs) below Iron Gate Dam from Jan 2012 to December 2012. Data collected after June 12, 2012 is provisional data. Data acquired from [USGS waterdata.usgs.gov](http://waterdata.usgs.gov).

No manipulated pulse flows occurred in Spring of 2012 as was the case in February 2011 (Figure 26). Iron Gate Dam did release water on August 31 2012 to increase base flows to a peak of 1,600 cfs, but this occurred after fish collections for the juvenile monitoring project were complete. 2012 flows below Iron Gate Dam are similar in magnitude to historic observations for 2007 and 2008. Temporally, the highest flows occurred slightly later in 2012 (April to mid-May) than in 2007 and 2008 where highest flows were generally in early Spring (February to April).

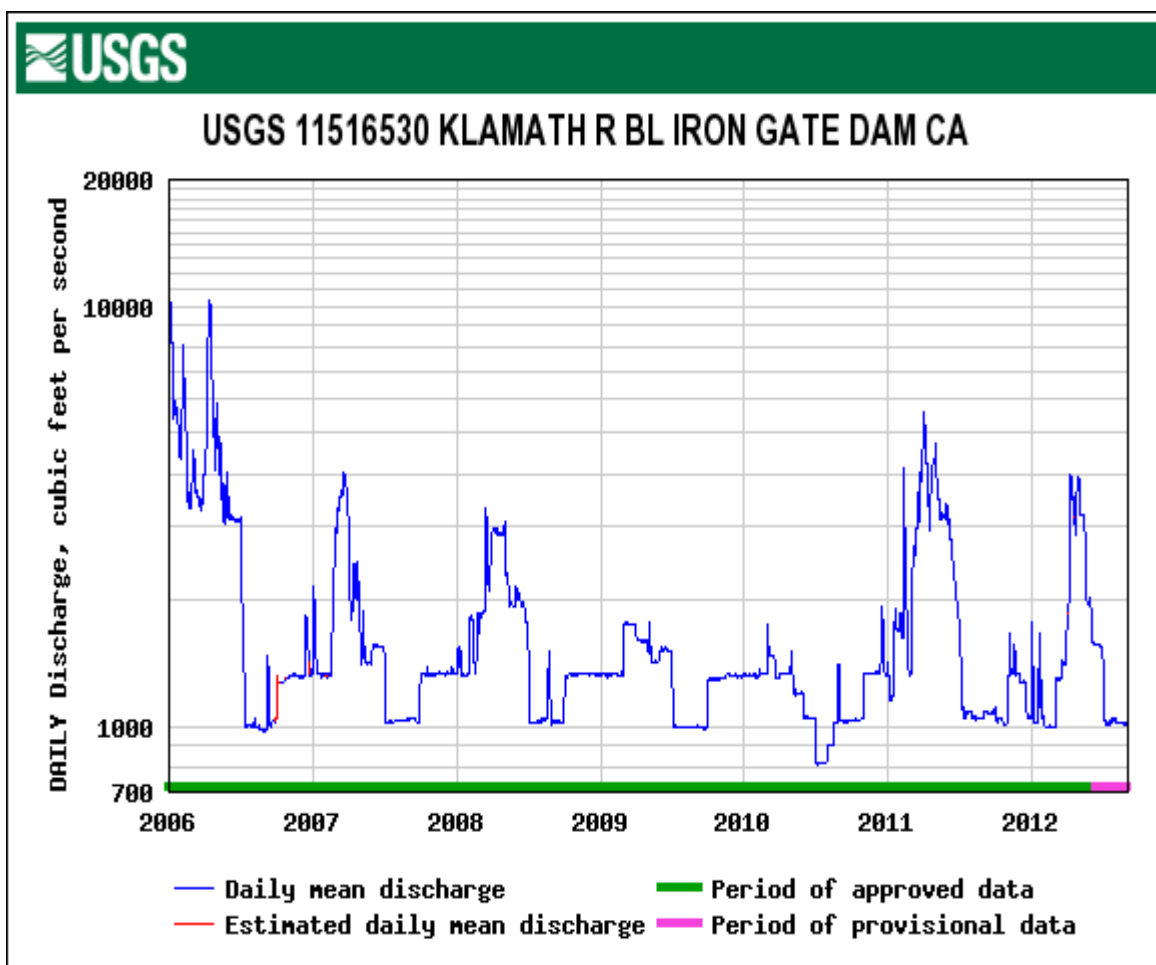


Figure 26. Daily discharge (log cfs) below Iron Gate Dam 2006-2012. Data acquired from USGS [waterdata.usgs.gov](http://waterdata.usgs.gov). Data collected after June 12, 2012 is provisional data.

## DISCUSSION

The annual prevalence of *C. shasta* and *P. minibicornis* infections in juvenile Chinook salmon have been monitored in fish health studies in the Klamath River since 2004 (Nichols and Foott 2006; Nichols et al. 2007; Nichols and True 2007; Nichols et al. 2008, True et al 2010). The monitoring program was standardized in 2006 to sample natural and mixed origin juvenile Chinook salmon in specific reaches of the Klamath River as fish are actively migrating downstream and then target CWT Chinook salmon in the lower reaches and Estuary. *Ceratomyxa shasta* prevalence of infection as determined by QPCR, in Chinook salmon captured above the Trinity River confluence during the peak migration period, is the annual metric used to compare disease prevalence in the Klamath River between monitoring study years. Prior to 2009, histological assessments, with the same data restrictions, were used.

Infectivity patterns of ceratomyxosis are well defined for native Klamath basin salmonid species. At river temperatures commonly observed (17-24°C) in the Klamath River during peak juvenile Chinook salmon migration, clinical disease occurs within three weeks of initial exposure resulting in moderate to high levels of mortality. This infectivity pattern has been established through sentinel susceptibility studies (Bartholomew 2010, Bjork and Bartholomew 2010, Stone et al. 2008, True et al. 2012) and annual monitoring of CWT Chinook salmon with known exposure periods in the main stem Klamath (Nichols and Foott 2006, Nichols et al. 2007, Nichols and True 2007, Nichols et al. 2009, True et al. 2010). This infectivity pattern is usually apparent in the majority of reaches as a bimodal distribution in bi-weekly prevalence of infection data: natural Chinook salmon sampled prior to hatchery releases, and in mixed-origin Chinook salmon collected from June to August. A similar temporal pattern for *C. shasta* is also frequently observed in Iron Gate Hatchery CWT Chinook salmon, WAL data, particularly for fish collected from the Estuary. Fish captured after 3-4 WAL have a high *C. shasta* POI and infectious load, and then a second peak in prevalence and DNA copy number occurs in groups of fish residing for longer periods of time. The timing of the second peak has varied from 5-9 WAL in the past two study years (Bolick et al. 2011, True et al. 2010). Therefore quantity of parasite DNA (infectious load), complimentary histological assessments of tissue damage, and CWT WAL data provide a degree of temporal and spatial information regarding annual prevalence of infection and disease severity in the mixed group of natural and hatchery origin juvenile out-migrants in the Klamath River.

Historically, the POI for *C. shasta* decreases in fish sampled below the Trinity River confluence. This is due in part to lower disease incidence in the Trinity River Chinook salmon entering the Klamath, as well as accretion of Trinity River flows into the lower reaches (K1 and K0). In 2012, we observed a higher *C. shasta* prevalence of infection in mixed-origin Chinook salmon sampled in this reach, compared to the reach above (Salmon to Trinity – K2) and the reach below (Estuary – K0). It is interesting to note that actinospore concentration, determined by water sampling conducted by OSU near the mouth of Tully Creek (RM38), has also increased in this lower reach in the past two years compare to other water sampling sites (Hallett 2011, Hallett 2012). Oregon State University researchers conducted a longitudinal survey in 2012, but did not detect a point source near the mouth of Tully Creek or marked differences in actinospore concentration above or below this site (Hallett 2012). It's difficult to know if this apparent increase in spore concentration in this lower reach is a true trend, or simply an artifact of increased sampling effort in this section of the lower Klamath River main stem.



### Annual Prevalence

In 2012, the annual *C. shasta* prevalence of infection (30%) above the Trinity confluence during peak migration was nearly double values observed for the past two years (17% in both 2010 and 2011). However 2010 and 2011 were extremely favorable environmental years in terms of cooler water temperatures, slower parasite development and therefore reduced parasite exposure in juvenile Chinook salmon. Environmental conditions in the Klamath River in 2012, in regard to elevated temperatures in the reaches below Iron Gate Dam in late April to late May (Figure 23), were more representative of conditions observed in 2006-2009. Despite well-defined infection patterns for Klamath River salmonids, predictions for quantitative myxozoan disease impacts at the population level are difficult to make in an actively emigrating juvenile Chinook population particularly when environmental factors such as river temperature and flows influence both parasite abundance and disease severity. How these factors influence parasite development, and disease progression in juvenile fish was discussed in detail in the 2011 annual report (Bolick et al. 2012).

### Klamath River Mixed Origin Chinook

Overall, 2012 was intermediate in ceratomyxosis disease prevalence and infection levels, compared to historical annual prevalence of infection observed from 2006- 2012 (Table 5, Page 28).

*Ceratomyxa shasta* prevalence of infection was low in natural fish sampled early in the season in upper reaches. We noted *C. shasta* was not detected in natural Chinook salmon sampled in the Shasta to Scott (K4) reach in April, and remained low and stable at 13% throughout May. After IGH releases on June 6, *C. shasta* POI was higher in the mixed origin and CWT groups, ranging from 10-20% through early July. Overall however, *C. shasta* POI for mixed origin groups in the Shasta to Scott (K4) reach was less than 10%.

As expected with increasing temperatures coupled with longer exposure periods later in the season, we observed increasing *C. shasta* POI in the Scott to Salmon (K3) reach in June and July. Prevalence of infection rose steadily from below 20% post IGH release (June 6-15) to a peak of 80% a month later (July 8) in mixed origin Chinook salmon. In 2012, *P. minibicornis* POI also reached typically high levels (over 90%) throughout July in the Scott to Salmon (K3) reach.

In the Salmon to Trinity (K2) reach, we observed bimodal peaks, with *C. shasta* POI of 58% on June 17 and a second peak of 68% on July 8. Based on infectivity patterns for *C. shasta*, it is likely these peaks represent infection prevalences in fish that migrate at different rates. For example, both groups were initially infected in the upper reaches but larger smolts likely emigrated rapidly while smaller Chinook salmon resided for a longer period of time (Wallace 2003). These later emigrants would be recaptured in the lower reaches at a later time, following a longer residency and likely prolonged exposure period.

In the Trinity to Estuary (K1) reach in mixed origin Chinook salmon, *C. shasta* POI was bell shaped with a rapid increase by July 1 that remained high (70-80%) throughout the sampling period ending mid-August. Whereas in the Estuary, where primarily IGH and TRH CWT Chinook salmon were targeted, there is a bimodal *C. shasta* POI of 60-70% in fish collected in mid-June to mid-July, and a smaller POI peak in fish collected in August (approximately 20-40%).

In summary, *C. shasta* POI in mixed origin groups (natural, unmarked and marked hatchery fish) by reach was expectedly low in the Shasta to Scott (K4). Following IGH releases, *C. shasta* POI rose to 36-37% in the Scott to Salmon (K3) and Salmon to Trinity (K2) reaches as these groups were tracked downstream. *Ceratomyxa shasta* POI was highest in the mixed origin group in the Trinity to Estuary

reach at 45% and in the Estuary at 39%. The higher *C. shasta* POI in mixed-origin fish collected in the lower river reaches is a function of disease progression from initial infection and/or repeated exposure in the upper reaches and subsequent development of ceratomyxosis disease approximately 3 weeks post exposure (True et al. 2012)..

#### IGH CWT Chinook

In the monitoring program, temporal data is derived from IGH CWT Chinook salmon, with known exposure periods based on hatchery release and in-river recapture dates (Weeks At Large).

As IGH CWT Chinook salmon were released from June 6-15, they encountered slightly warmer temperatures and spore densities compared to natural fish. Spore densities in the infectious zone (Kinsman trap, Figure 5) began to reach the lower infection threshold associated with disease and mortality (1 spores/L, personal communication S. Hallett, OSU) on May 20 - prior to hatchery releases. Typically IGH releases Chinook salmon in mid-May (2005-2007, and 2009), but this is generally driven by target size at release as well as favorable river temperatures. Fish can be released approximately 3-4 weeks later in some years (2008, 2010, and 2012).

Overall, *C. shasta* POI in IGH CWT Chinook salmon was 37% for juveniles captured above the Trinity River confluence, 47% below the confluence, and 39% in the Estuary. As ceratomyxosis would not be expected to be decreasing over time, the decrease in *C. shasta* POI in the Estuary compared to the reach above likely indicates some proportion of severely infected fish were dropping out of the sample population. *Parvicapsula minibicornis* in all IGH CWT Chinook salmon tested in late June to late August was 89% and 96% in IGH CWT collected below the Trinity River confluence.

For WAL analysis of IGH CWT juvenile Chinook salmon, we observed the highest *C. shasta* POI in fish captured after residing for 3 weeks post release, again the typical period for development of ceratomyxosis at the river temperatures observed in the Klamath in 2012 (approximately 17-19 °C below IGD and 15-19 °C at the Seiad Valley gauge). In terms of parasite infectious load, measured as quantity of *C. shasta* DNA present in intestinal tissue, IGH CWT Chinook had much higher parasite loads in 2012 compare to 2011. As stated earlier, 2011 had favorable environmental conditions, and parasite load in juvenile Chinook salmon was between 1000-2000 DNA copies in infected CWT Chinook salmon at 3-5 WAL. In 2012, both *C. shasta* prevalence of infection and mean DNA quantity observed in IGH CWT Chinook followed a bell shaped curve for groups residing between less than 1 to 5 WAL. A second peak in parasite mean DNA copy number occurred in fish residing 7 WAL, despite an decreased *C. shasta* POI (less than 20%) in this group. Specifically, parasite load in IGH CWT captured after residing for 3 WAL was 60,000 DNA copies and 120,000 copies in the 7 WAL group. These DNA quantities indicate that while fewer fish were infected in the 7 WAL group, the Chinook salmon that were infected had heavier *C. shasta* parasite infection levels than fish captured after residing for 3 WAL. The majority of CWT captured in the lower reaches and Estuary were residing 3-4 WAL (N=100) and likely represent the fastest migration group (smolt ready fish released from IGH, Wallace 2003). Groups residing for longer periods of time likely reared for some period in the upper reaches before being captured at 6-9 WAL in the lowest reach (Trinity to Estuary) or in the Estuary.

#### Trinity River Mixed Origin Chinook

For the Trinity River, prevalence of infection for all juvenile Chinook salmon collected in the Trinity River was 4% for *C. shasta* and 24% for *P. minibicornis*. Specifically, *C. shasta* POI was 2% (N=117) in fish collected from the upper Trinity (T2) site, and increased to 6% (N=120) in fish collected from the lower Trinity (T1) site near the confluence with the Klamath River.

### TRH CWT Chinook

Temporal data for TRH CWT compares *C. shasta* prevalence of infection in mixed origin Chinook salmon collected within the Trinity River and TRH CWT Chinook captured within the Trinity and then in the Klamath River, including the Estuary. As discussed above, *C. shasta* POI was low in mixed origin Chinook salmon in the Trinity River, but did increase from 2-6% from the upper to lower reaches. In TRH CWT Chinook specifically, *C. shasta* POI increased from 3% in fish collected at the upper Pear Tree site to 10% at the lower Willow Creek site. These similar POI values in both Trinity mixed origin and CWT Chinook salmon indicate juvenile fish were exposed and lightly infected with *C. shasta* prior to entering the Klamath River. In the Klamath River only five TRH CWT Chinook were captured in the Trinity to Estuary (K1) reach, *C. shasta* POI was 40% in this small sample set. In the Estuary, TRH CWT *C. shasta* POI was similar at 43% in the 49 fish sampled. In terms of parasite load, expressed as *C. shasta* mean DNA copy number in intestinal tissue, infection levels were low in TRH CWT sampled in the Trinity River. The mean DNA copy number was near the assay detection limit at 10 copies for the Pear Tree site, and 4 copies at the lower Willow Creek site. In the two *C. shasta* positive TRH CWT collected from the Trinity to Estuary reach, the mean DNA copy number was 24. Only in TRH CWT collected from the Estuary did we see an increase in the magnitude of *C. shasta* DNA present. DNA copy number in this group remained low from June 20 to July 25, however TRH CWT sampled in August had DNA levels comparable to IGH CWT Chinook sampled from the Estuary. It is interesting to note that mean copy number in TRH CWT sampled in the Estuary (32,800 copies) was not markedly different from levels observed in IGH CWT (39,600) sampled over the exact same time period. This raises additional questions about parasite exposure in the Trinity to Estuary reach. To put these DNA levels in perspective, in the 2008 Prognosis study *C. shasta* mean DNA copy number in TRH Chinook (exposed for 72 hours at Beaver Creek) was well over 6 logs at 10-15 days post exposure and over 8 logs at the mean death to death, which was 21 days post exposure (True et al. 2012). Based on the release date of June 1-15, TRH CWT Chinook sampled from the Estuary in 2012 (June 20 through August 23) would have been residing approximately 2-12 weeks post release and had parasite DNA levels of approximately 4 logs.

Exposure to *P. minibicornis* resulting in low level infections was also observed in TRH CWT Chinook salmon sampled in the Trinity River: 40% (mean copy number 189) at the upper Pear Tree site and 36% (mean copy number 165) at the Willow Creek site. *Parvicapsula minibicornis* mean DNA copy number was also quite low (mean copy number 251) in TRH CWT Chinook salmon sampled in the Estuary. DNA levels of this low magnitude indicate exposure rather than active or clinical infections. Again, compared to DNA levels observed in the 2008 Prognosis study, *P. minibicornis* mean DNA copy number in TRH Chinook was over 6 logs at mean day to death, when fish succumbed to ceratomyxosis (rather than glomerulonephritis and osmotic imbalance caused by clinical disease with *P. minibicornis*).

### Summary

The higher *C. shasta* prevalence of infection observed in mixed origin Chinook salmon in the Klamath River in 2012 was likely due to warmer water temperatures (~3-4 °C) in April through June observed in the infectious zone, compared to the past two years of the monitoring program. Higher temperatures in the infectious zone allow faster development of polychaete worms infected with *C. shasta*, and subsequently earlier maturation and/or release of infectious actinospores into the water column. Natural Chinook salmon appeared to have lower *C. shasta* prevalence of infection than IGH Chinook salmon in 2012. However prevalence of infection for natural Chinook salmon sampled later in the season is typically masked by the lower POI observed in mixed-origin fish collected after IGH hatchery releases. Therefore it's difficult to discern if lower *C. shasta* POI in natural Chinook salmon is due to sampling

period (cooler temperatures) or actually lower infectivity associated with an earlier migration period out of the upper reaches. With higher temperatures in 2012, IGH CWT Chinook salmon expectedly had higher *C. shasta* POI (42%) and parasite infectious load compared to the past two monitoring years, but annual POI for mixed origin Chinook during the peak migration period (above the Trinity River) was relatively moderate (30%) in relationship to the historical annual metric. Similarly, *P. minibicornis* annual prevalence of infection (69%) in mixed origin Chinook salmon was intermediate in 2012 when compared to studies conducted from 2005 to present.

The majority of juvenile Chinook examined appeared relatively healthy: some clinical disease was observed during QPCR necropsy and by histology but in relatively few fish. *Parvicapsula minibicornis* annual prevalence of infection was similarly intermediate in mixed origin juvenile Chinook salmon, and followed a general trend of increasing and sustained high prevalence throughout the migration period.

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**Joe Polos, USFWS Arcata FWO, Supervisory Fish Biologist**  
**Mike Belchik, Yurok Tribe Fisheries, Senior Biologist, Klamath River Division**  
**Robert Ray, PhD Student, Oregon State University, Dept. of Fisheries and Wildlife**

### **Author Roles**

The contributions of each author have been summarized below.

- Kimberly True – Project coordination, data management and quality control, QPCR methods and quality assurance, data analysis and written report.
- Anne Bolick – Data management and quality control, QPCR necropsy extraction and assays, pivot tables and environmental data figures, and compilation of reviewers' comments.
- Scott Foott – Project support, examination of histological specimens, diagnostic assessments, histological report sections, and editorial review.

## APPENDIX A – Histological Summary Table

**Table A1. Parasite prevalence of infection [number positive / total (%)], pathology score for kidney and intestine, and tissue abnormalities observed in histological sections of juvenile Klamath River Chinook salmon collected from the Shasta to Scott reach (K4). Collection dates are reported as Monday of given week.**

Collection week	Apr 29	May 13	May 27	Jun 10	Jun 24	POI
<b>Kidney</b>						
Pm Troph.	1 / 10 (10)	2 / 10 (20)	6 / 10 (60)	9 / 9 (100)	4 / 9 (44)	22 / 48 (46)
Pm Myxosp.	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 9 (0)	0 / 9 (0)	0 / 48 (0)
Metacercaria	0 / 10 (0)	2 / 10 (20)	1 / 10 (10)	0 / 9 (0)	0 / 9 (0)	3 / 48 (6)
<i>C. shasta</i> troph.	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 9 (0)	0 / 9 (0)	0 / 48 (0)
<i>Chloromyxum</i> sp	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 9 (0)	0 / 9 (0)	0 / 48 (0)
<b>Pathology Score</b>	0.00	0.00	0.30	4.11	0.33	
<b>Intestinal tract</b>						
<i>C. shasta</i> troph.	0 / 10 (0)	2 / 10 (2)	1 / 9 (11)	1 / 10 (10)	0 / 10 (0)	4 / 49 (8)
<i>C. shasta</i> myxosp.	0 / 10 (0)	0 / 10 (0)	0 / 9 (0)	0 / 10 (0)	0 / 10 (0)	0 / 49 (0)
Helminth	0 / 10 (0)	0 / 10 (0)	1 / 9 (11)	0 / 10 (0)	0 / 10 (0)	1 / 49 (2)
<b>Pathology Score</b>	0.00	0.00	0.00	0.00	0.00	
Adipose steatitis	0 / 8 (0)	6 / 8 (75)	1 / 3 (33)	2 / 6 (33)	5 / 6 (83)	14 / 31 (45)
Adipose lipofuscin	0 / 8 (0)	0 / 8 (0)	0 / 3 (0)	0 / 6 (0)	3 / 6 (50)	3 / 31 (10)
<b>Gill</b>						
Ich	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 50 (0)
Glochidia	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 50 (0)
Miracidia	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 50 (0)
Metacercaria	0 / 10 (0)	5 / 10 (50)	6 / 10 (60)	10 / 10 (100)	7 / 10 (70)	28 / 50 (56)
Invasive <i>C. shasta</i>	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 50 (0)
Multif. Hyperplasia	0 / 10 (0)	4 / 10 (40)	4 / 5 (80)	10 / 10 (100)	5 / 10 (50)	23 / 45 (51)

**Table A2. Parasite prevalence of infection [number positive / total (%)], pathology score for kidney and intestine, and tissue abnormalities observed in histological sections of juvenile Klamath River Chinook salmon collected from the Scott to Salmon River (K3). Collection dates are reported as Monday of given week.**

Collection Week	Jun 17	Jul 1	Jul 15	POI
<u>Kidney</u>				
Pm Troph.	6 / 10 (60)	9 / 9 (100)	10 / 10 (100)	25 / 29 (86)
Pm Myxosp.	0 / 10 (0)	0 / 9 (0)	0 / 10 (0)	0 / 29 (0)
Metacercaria	4 / 10 (40)	0 / 9 (0)	2 / 10 (20)	6 / 29 (21)
<i>C. shasta</i> troph.	0 / 10 (0)	0 / 9 (0)	0 / 10 (0)	0 / 29 (0)
<i>Chloromyxum</i> sp	1 / 10 (10)	0 / 9 (0)	0 / 10 (0)	1 / 29 (3)
<b>Pathology Score</b>	2.3	4.1	6.3	
<u>Intestinal tract</u>				
<i>C. shasta</i> troph.	1 / 10 (10)	1 / 10 (10)	0 / 10 (0)	2 / 30 (7)
<i>C. shasta</i> myxosp.	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 30 (0)
Helminth	2 / 10 (20)	0 / 10 (0)	0 / 10 (0)	2 / 30 (7)
<b>Pathology Score</b>	0.00	0.14	0.00	
Adipose steatitis	3 / 7 (43)	2 / 6 (33)	8 / 8 (100)	13 / 21 (62)
Adipose lipofuscin	0 / 7 (0)	0 / 6 (0)	3 / 8 (38)	3 / 21 (14)
<u>Gill</u>				
Ich	0 / 9 (0)	0 / 10 (0)	0 / 10 (0)	0 / 29 (0)
Glochidia	0 / 9 (0)	0 / 10 (0)	0 / 10 (0)	0 / 29 (0)
Miracidia	0 / 9 (0)	0 / 10 (0)	0 / 10 (0)	0 / 29 (0)
Metacercaria	7 / 9 (77)	10 / 10 (100)	9 / 10 (90)	28 / 29 (90)
Invasive <i>C. shasta</i>	0 / 9 (0)	0 / 10 (0)	0 / 10 (0)	0 / 29 (0)
Multif. Hyperplasia	6 / 9 (66)	10 / 10 (100)	9 / 10 (90)	25 / 29 (86)

**Table A3. Parasite prevalence of infection [number positive / total (%)], pathology score for kidney and intestine, and tissue abnormalities observed in histological sections of juvenile Klamath River Chinook salmon collected from the Salmon to Trinity River reach (K2). Collection dates are reported as Monday of given week.**

Collection Week	Jun 17	Jul 1	Jul 15	POI
<u>Kidney</u>				
Pm Troph.	9 / 10 (90)	10 / 10 (100)	6 / 10 (60)	25 / 30 (83)
Pm Myxosp.	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 30 (0)
Metacercaria	3 / 10 (30)	1 / 10 (10)	3 / 10 (30)	7 / 30 (23)
<i>C. shasta</i> troph.	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 30 (0)
<i>Chloromyxum</i> sp	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 30 (0)
<b>Pathology Score</b>	1.5	2.6	2.3	
<u>Intestinal tract</u>				
<i>C. shasta</i> troph.	0 / 10 (0)	1 / 9 (11)	2 / 10 (20)	3 / 29 (10)
<i>C. shasta</i> myxosp.	0 / 10 (0)	0 / 9 (0)	0 / 10 (0)	0 / 29 (0)
Helminth	0 / 10 (0)	0 / 9 (0)	1 / 10 (10)	1 / 29 (3)
<b>Pathology Score</b>	0.00	0.00	0.90	
Adipose steatitis	3 / 4 (75)	8 / 10 (80)	5 / 9 (56)	16 / 23 (70)
Adipose lipofuscin	0 / 4 (0)	1 / 10 (10)	1 / 9 (11)	2 / 23 (7)
<u>Gill</u>				
Ich	0 / 10 (0)	0 / 10 (0)	1 / 10 (10)	1 / 30 (3)
Glochidia	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 30 (0)
Miracidia	0 / 10 (0)	0 / 10 (0)	1 / 10 (10)	1 / 30 (3)
Metacercaria	10 / 10 (100)	9 / 10 (90)	9 / 10 (90)	28 / 30 (93)
Invasive <i>C. shasta</i>	0 / 10 (0)	0 / 10 (0)	1 / 10 (10)	1 / 30 (3)
Multif. Hyperplasia	10 / 10 (100)	10 / 10 (100)	10 / 10 (100)	30 / 30 (100)



**Table A4. Parasite prevalence of infection [number positive / total (%)], pathology score for kidney and intestine, and tissue abnormalities observed in histological sections of juvenile Klamath River Chinook salmon collected from the Trinity to Estuary reach (K1). Collection dates are reported as Monday of given week.**

Collection Week	Jun 17	Jul 1	Jul 15	Aug 5	POI
<u>Kidney</u>					
Pm Troph.	9 / 9 (100)	8 / 10 (80)	7 / 10 (70)	8 / 10 (80)	32 / 39 (82)
Pm Myxosp.	0 / 9 (0)	0 / 10 (0)	1 / 10 (10)	1 / 10 (10)	2 / 39 (5)
Metacercaria	5 / 9 (55)	0 / 10 (0)	2 / 10 (20)	2 / 10 (20)	9 / 39 (23)
<i>C. shasta</i> troph.	0 / 9 (0)	0 / 10 (0)	1 / 10 (10)	0 / 10 (0)	1 / 39 (3)
<i>Chloromyxum</i> sp	0 / 9 (0)	0 / 10 (0)	1 / 10 (10)	0 / 10 (0)	1 / 39 (3)
<b>Pathology Score</b>	0.33	0.80	3.6	3.6	
<u>Intestinal tract</u>					
<i>C. shasta</i> troph.	1 / 10 (10)	0 / 10 (0)	1 / 10 (10)	0 / 10 (0)	2 / 40 (5)
<i>C. shasta</i> myxosp.	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 40 (0)
Helminth	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	1 / 10 (10)	1 / 40 (3)
<b>Pathology Score</b>	0.00	0.00	0.30	0.30	
Adipose steatitis	6 / 10 (60)	9 / 10 (90)	5 / 5 (100)	3 / 7 (43)	23 / 32 (72)
Adipose lipofuscin	1 / 10 (10)	2 / 10 (20)	0 / 5 (0)	1 / 7 (14)	4 / 32 (13)
<u>Gill</u>					
Ich	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	1 / 10 (10)	1 / 40 (3)
Glochidia	1 / 10 (10)	0 / 10 (0)	1 / 10 (0)	0 / 10 (0)	2 / 40 (5)
Miracidia	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 40 (0)
Metacercaria	8 / 10 (80)	6 / 10 (60)	7 / 10 (70)	6 / 10 (60)	27 / 40 (68)
Invasive <i>C. shasta</i>	0 / 10 (0)	0 / 10 (0)	1 / 10 (10)	0 / 10 (0)	1 / 40 (3)
Multif. Hyperplasia	8 / 10 (80)	8 / 10 (80)	6 / 10 (60)	8 / 10 (80)	30 / 40 (75)

**Table A5. Parasite prevalence of infection [number positive / total (%)], pathology score for kidney and intestine, and tissue abnormalities observed in histological sections of juvenile Klamath River Chinook salmon collected from Upper Trinity River (T2) rotary screw trap at Pear tree trap (RM 94). Collection dates are reported as Monday of given week.**

Collection Week	May 27	Jul 1	POI
<u>Kidney</u>			
Pm Troph.	0 / 8 (0)	0 / 10 (0)	0 / 18 (0)
Pm Myxosp.	0 / 8 (0)	0 / 10 (0)	0 / 18 (0)
Metacercaria	1 / 8 (13)	0 / 10 (0)	1 / 18 (6)
<i>C. shasta</i> troph.	0 / 8 (0)	0 / 10 (0)	0 / 18 (0)
<i>Chloromyxum</i> sp	2 / 8 (25)	0 / 10 (0)	2 / 18 (11)
<b>Pathology Score</b>	0.00	0.60	
<u>Intestinal tract</u>			
<i>C. shasta</i> troph.	0 / 9 (0)	0 / 10 (0)	0 / 19 (0)
<i>C. shasta</i> myxosp.	0 / 9 (0)	0 / 10 (0)	0 / 19 (0)
Helminth	0 / 9 (0)	0 / 10 (0)	0 / 19 (0)
<b>Pathology Score</b>	0.00	0.00	
Adipose steatitis	0 / 4 (0)	2 / 8 (25)	2 / 12 (17)
Adipose lipofuscin	0 / 4 (0)	1 / 8 (13)	1 / 12 (8)
<u>Gill</u>			
Ich	0 / 8 (0)	0 / 10 (0)	0 / 18 (0)
Glochidia	0 / 8 (0)	5 / 10 (50)	5 / 18 (28)
Miracidia	0 / 8 (0)	0 / 10 (0)	0 / 18 (0)
Metacercaria	0 / 8 (0)	0 / 10 (0)	0 / 18 (0)
Invasive <i>C. shasta</i>	0 / 8 (0)	0 / 10 (0)	0 / 18 (0)
Multif. Hyperplasia	0 / 8 (0)	2 / 10 (20)	2 / 18 (11)

**Table A6. Parasite prevalence of infection [number positive / total (%)], pathology score for kidney and intestine, and tissue abnormalities observed in histological sections of juvenile Klamath River Chinook salmon collected from the Lower Trinity River (T1) rotary screw trap at Willow Creek trap (RM 14). Collection dates are reported as Monday of given week.**

Collection Week	May 27	Jul 1	POI
<u>Kidney</u>			
Pm Troph.	0 / 9 (0)	0 / 7 (0)	0 / 16 (0)
Pm Myxosp.	0 / 9 (0)	0 / 7 (0)	0 / 16 (0)
Metacercaria	0 / 9 (0)	1 / 7 (14)	1 / 16 (6)
<i>C. shasta</i> troph.	0 / 9 (0)	0 / 7 (0)	0 / 16 (0)
<i>Chloromyxum</i> sp	2 / 9 (22)	2 / 7 (29)	4 / 16 (25)
<b>Pathology Score</b>	0.00	0.29	
<u>Intestinal tract</u>			
<i>C. shasta</i> troph.	0 / 10 (0)	0 / 3 (0)	0 / 13 (0)
<i>C. shasta</i> myxosp.	0 / 10 (0)	0 / 3 (0)	0 / 13 (0)
Helminth	0 / 10 (0)	0 / 3 (0)	0 / 13 (0)
<b>Pathology Score</b>	0.00	0.00	
Adipose steatitis	0 / 0 (0)	0 / 1 (0)	0 / 1 (0)
Adipose lipofuscin	0 / 0 (0)	0 / 1 (0)	0 / 1 (0)
<u>Gill</u>			
Ich	0 / 9 (0)	0 / 3 (0)	0 / 12 (0)
Glochidia	0 / 9 (0)	1 / 3 (33)	1 / 12 (8)
Miracidia	0 / 9 (0)	0 / 3 (0)	0 / 12 (0)
Metacercaria	0 / 9 (0)	1 / 3 (33)	1 / 12 (8)
Invasive <i>C. shasta</i>	1 / 9 (11)	1 / 3 (0)	2 / 12 (17)
Multif. Hyperplasia	0 / 9 (0)	1 / 1 (100)	1 / 10 (10)

**APPENDIX B – Summary table of *Ceratomyxa shasta* infection by QPCR in juvenile Chinook salmon sampled from 5 reaches within the Klamath River and the upper and lower Trinity River. The prevalence (#positive/#sampled) is presented for each sample reach by collection week and sample date.**

<b>Collection Week</b>	<b>Sample Date</b>	<b>Shasta R. to Scott R.</b>	<b>Scott R. to Salmon R.</b>	<b>Salmon R. to Trinity R.</b>	<b>Trinity R. to Estuary</b>	<b>Estuary</b>	<b>Upper Trinity R.</b>	<b>Lower Trinity R.</b>
1	1-Apr							
2	8-Apr	0% (0/15)						
3	15-Apr	0 % (0/15)						
4	22-Apr	0 % (0/15)						
5	29-Apr	0% (0/15)	0% (0/15)	0% (0/15)	0% (0/15)		0% (0/15)	0% (0/15)
6	6-May	13 % (2/15)						
7	13-May	13% (2/15)	0% (0/15)	0% (1/15)	0% (0/15)		0% (0/15)	0% (0/15)
8	20-May	7% (1/15)						
9	27-May	13% (2/15)	7% (1/15)	0% (1/15)	7% (1/15)		0% (0/15)	0% (0/15)
10	3-Jun	0% (0/14)						
11	10-Jun	20% (4/20)	15% (3/20)	45% (9/20)				
12	17-Jun	10% (2/20)	43% (9/21)	57% (12/21)	25% (5/20)	67% (2/3)	5% (1/20)	0% (0/20)
13	24-Jun	15% (3/20)	25% (5/20)	35% (7/20)		55% (11/20)		
14	1-Jul	20% (4/20)	48% (19/40)	40% (8/20)	71% (15/21)	35% (7/20)	0% (0/12)	0% (0/18)
15	8-Jul	9% (1/11)	80% (35/44)	75% (15/20)		75% (15/20)		
16	15-Jul		25% (5/20)	55% (11/20)	80% (16/20)	70% (14/20)	5% (1/20)	10% (2/20)
17	22-Jul					20% (4/20)		
18	29-Jul				70% (14/10)	10% (2/20)	0% (0/20)	29% (5/17)
19	5-Aug					20% (4/20)		
20	12-Aug				75% (15/20)	20% (4/20)		
21	19-Aug					40% (8/20)		
		<b>K4 Total 9% (21/225)</b>	<b>K3 Total 37% (77/210)</b>	<b>K2 Total 37% (62/166)</b>	<b>K1 Total 45% (66/146)</b>	<b>K0 Total 39% (71/183)</b>	<b>T2 Total 2% (2/117)</b>	<b>T1 Total 6% (7/120)</b>

**APPENDIX C – Summary table of *Parvicapsula minibicornis* infection by QPCR in juvenile Chinook salmon sampled from 5 reaches within the Klamath River and lower Trinity River. The prevalence (#positive/#sampled) is presented for each sample reach by collection week and sample date.**

<b>Collection Week</b>	<b>Sample Date</b>	<b>Shasta R. to Scott R.</b>	<b>Scott R. to Salmon R.</b>	<b>Salmon R. to Trinity R.</b>	<b>Trinity R. to Estuary</b>	<b>Estuary</b>	<b>Upper Trinity R.</b>	<b>Lower Trinity R.</b>
1	1-Apr							
2	8-Apr	0% (0/15)						
3	15-Apr	0% (0/15)						
4	22-Apr	7% (1/15)						
5	29-Apr	0% (0/15)	7% (1/15)	0% (0/15)	7% (1/15)		0% (0/15)	0% (0/15)
6	6-May	13% (2/15)						
7	13-May	60% (9/15)	0% (0/15)	13% (2/15)	60% (9/15)		0% (0/15)	0% (0/15)
8	20-May	73% (11/15)						
9	27-May	87% (13/15)	67% (10/15)	67% (10/15)	13% (2/15)		13% (2/15)	0% (0/15)
10	3-Jun	93% (13/14)						
11	10-Jun	0% (0/20)						
12	17-Jun	55% (11/20)				0% (0/3)	70% (14/20)	40% (8/20)
13	24-Jun	65% (13/20)				65% (13/20)		
14	1-Jul	100% (20/20)	96% (25/26)		95% (20/21)	60% (12/20)	25% (3/12)	28% (5/18)
15	8-Jul	100% (11/11)	100% (28/28)	100% (5/5)		95% (19/20)		
16	15-Jul		100% (20/20)	100% (19/20)	90% (18/20)	95% (19/20)	40% (8/20)	40% (8/20)
17	22-Jul					90% (18/20)		
18	29-Jul				95% (19/20)	85% (17/20)	10% (2/20)	41% (7/18)
19	5-Aug					95% (19/20)		
20	12-Aug				90% (18/20)	100% (20/20)		
21	19-Aug					90% (18/20)		
		<b>K4 Total 46% (104/225)</b>	<b>K3 Total 71% (84/119)</b>	<b>K2 Total 51% (36/70)</b>	<b>K1 Total 69% (87/126)</b>	<b>K0 Total 85% (155/183)</b>	<b>T2 Total 25% (29/117)</b>	<b>T1 Total 23% (28/120)</b>

## APPENDIX E - Reviewers' comments

Listed below are verbatim (in quotes) or paraphrased comments provided by reviewers of a draft of this report. The primary author's reply is given unless noted otherwise (additional authors name and responses are provided for specific sections of this report).

### Reviewer #1

**Pg. 2 – Summary.** Seasonal prevalence above the Trinity River is reported at 30%, when in the results it is reported at 36.8%

Response: 30% is correct as it refers to Table 5 and data that has been restricted to collection dates between May and June.

**Pg. 2 – Summary.** In reference to the statement that higher *C. shasta* actinospore densities in 2012 resulted in an increase of annual infection prevalence, the reviewer stated that densities were pretty similar between 2011 and 2012.

Response: We referenced Hallett's Sept 2012 report where spore density at BC was higher earlier in the year (May) when juveniles were actively migrating. Specified this observation was for the earlier May period in the narrative.

**Pg. 8 – Methods.** In reference to terms used, the reviewer stated that we do not refer to annual prevalence in the results.

Response: Annual prevalence data is given in the results on page 28 when discussing historical data and annual *C. shasta* prevalence in Table 5.

**Pg. 9 – Results.** Reviewer wants to see the increase of spore density in water samples near Tully Creek addressed in the discussion.

Response: We referenced an OSU report stating that below the confluence the disease incidence has increased near Tully Creek over the past two years. This has been moved to the discussion.

**Pg. 11 – Results.** Reviewer would like to see Table 4 reorganized. "Move ALL CWT to the top of the section and then break it down further"

Response: Table organized in order of importance. IGH CWT Chinook listed first; followed by TRH CWT. ALL CWT doesn't hold as much weight as the hatchery CWT because ALL CWT includes unreadable tags. Therefore, we left the table in the existing order.

**Pg. 12 – Results.** In reference to the location of the Kinsman trap in the infectious zone, the reviewer states that saying it is at the upper end of the zone might be incorrect. Reviewer thinks it is more in the middle of the zone.

Response: We consider the upper reach from Iron Gate Dam to the Shasta (K5) to be in the infectious zone, however *C. shasta* POI historically has been low in this upper reach therefore we felt the important areas of the infectious zone are further downstream. We simplified the sentence to say the location was the Kinsman trap and we added a sentence about the infectious zone in the introduction.

**Pg. 13 – Results.** The reviewer asked if there is an annual metric for *P. minibicornis*.

Response: No. We consider *C. shasta* to be the significant pathogen for juvenile Chinook salmon. While *P. minibicornis* POI is quite high, this parasite is best evaluated histologically. Scott Foott developed the *P. minibicornis* pathology score to evaluate disease severity but it is not an annual metric.

**Pg. 27 – Results.** Reviewer suggests that we clarify that TRH POI is higher in K1 and K0, but higher than what?

Response: Sentence changed to reflect that *C. shasta* POI in K1 and K0 was higher than fish collected in the Trinity River.

**Pg. 28 – Results.** Reviewer questions whether the explanation of historic annual prevalence should be in the methods section instead.

Response: We think it is appropriate to leave the paragraph in the results as we are using it to explain the results in Table 5. We were explaining that histology was the screening method in the earlier years, but that has transitioned to QPCR in recent years. This paragraph is an introduction to the table.

**Pg. 29 – Results.** Reviewer suggests that we move the explanation of bimodal peaks to the discussion.

Response: This is repeated in detail in the discussion (Pg. 36); however we were just explaining briefly that the pattern of this graph is not unusual to see and the discussion goes further to depth about the bimodal peaks and why this occurs. We also feel some discussion in results sections is warranted due to the lengthy and technical aspects of the full report. We think it would be difficult for readers to follow the material if no discussion of results occurred until the end of the report. We summarize results and provide added more depth in the discussion section.

**Pg. 37 – Discussion.** At the top of this page we discuss this reach having a POI less than 10%. Reviewer would like us to clarify to which reach we are referring.

Response: Sentence changed to reflect the reach discussed is K4.

**Pg. 37 – Discussion.** Reviewer pointed out that we called the Trinity sites by a different name (upper or lower) in the results then we do in the discussion.

Response: Discussion changed to match results language.

**Pg. 37 – Discussion.** Reviewer states that data ( $C_T$  values) for natural fish from 2011 and 2012 was not reported in the results.

Response: We prefer to discuss infection levels in terms of parasite DNA copy number, rather than  $C_T$  values.  $C_T$  values are inversely related to parasite DNA copy number which can cause confusion in readers not familiar with this assay. DNA levels were not very different in natural fish between 2011 and 2012, so the sentence was removed rather than modified to copy number.

## Reviewer #2

**General comment.** Dates are presented in two formats (day Month and Month day) throughout the report. Select one format.

Response: All dates have been changed to month day format.

**General comment.** Many of the figures are missing axis labels.

Response: The majority of figures are prevalence of infection levels (y axis) by sampling period (x axis) are explained in detail in the chart caption and/or legend. The report has multiple authors and some figures, such as histological charts, are formatted differently because they were prepared by different authors.

**Pg. 3 – Introduction.** Reviewer suggests that the data we report from table 5 should be limited to 2006-2011 in the introduction and then 2012 data should be reported in the results section.

Response: We changed language for ‘historical data’ to exclude the current year results.

**Pg. 6 – Methods.** Reviewer asked if table 2 should include Trinity River sample data as well.

Response: Trinity River sampling dates/numbers were added to table.

**Pg. 7 – Methods.** We stated that *P. minibicornis* infections are generally high early in the season. The reviewer would like this to be moved to the results or discussion.

Response: This is in the methods section because we are explaining that due to high *P. minibicornis* infections only a subsample of kidney tissue was assayed by QPCR. Therefore, it is applicable to the methods section.

**Pg. 8 – Methods.** Reviewer suggests that annual prevalence should not describe infection for one calendar year. It should instead reflect the period that is sampled (spring/summer).

Response: Detailed definitions are given for point POI and period or annual POI on page 8. Language changed to clarify that annual prevalence is used to compare POI from year to year during the sampling period. For annual prevalence that period is May-July (Table 5).

**Pg. 9 - Results.** Reviewer would like to see a table for *P. minibicornis* similar to Table 2, which shows number of fish sampled by reach.

Response: Appendix C summarizes the *P. minibicornis* infection data by reach. *Ceratomyxa shasta* is the parasite that tends to drive mortality, and therefore we decided to only include the *C. shasta* table in the report itself. However, the *P. minibicornis* data is listed in this appendix.

**Pg. 10 - Results.** Reviewer wants clarification on why unmarked fish were not collected in the estuary, and if this skews the comparisons on infection trends.

Response: Unmarked fish were not collected in the estuary because that is not the study objective or design. We have collected unmarked fish in the estuary in past studies, and found the quality of data provided for this effort is low. Parasite POI in unknown fish (unknown natural or hatchery, and/or Klamath or Trinity or other tributary origin) does not provide as much clarity as fish with known origin and residency since hatchery release. Looking at CWT fish in the estuary gives us a much clear picture of the two hatchery Chinook salmon groups and their disease response in a given migration period and season.



**Pg. 10 - Results.** In reference to CWT salmon, the reviewer suggests removing the Trinity River sample numbers to a different section. Seems out of place

Response: Trinity River sample numbers were included in this section because we are discussing total number of CWT fish collected in 2012 which was 554. This number was mentioned earlier in the paper, and we wanted to show where that number came from. Trinity River Hatchery CWT Chinook salmon are discussed in detail in a later section of the report.

**Pg. 10 – Results.** In reference to mixed origin Chinook collected above and below the Trinity River confluence, the reviewer thinks that the estuary should not be included as unmarked fish were not collected in the estuary. Reviewer suggests leaving the estuary out when discussing above and below the confluence.

Response: We explained why the demarcation of the Trinity River (above and below the confluence) is important in discussing disease impacts in the lower Klamath basin. The accretion of Trinity River flows and co-mingling of uninfected (or low infection level) TRH Chinook salmon skews POI data in lower reaches (K1-Trinity to Estuary and K0-Estuary). This occurs in because the lower POI in TRH Chinook salmon dilutes the overall parasite POI observed in both lower reaches and could erroneously be attributed to decreasing disease in the overall sample groups. We added language to describe the proportion of natural, MOC and CWT Chinook sampled in K1 compared to only CWT Chinook sampled in the Estuary.

**Pg. 12 – Results.** This is the first time that the reader is introduced to the infectious zone. The reviewer thinks we need to elaborate on this topic or bring it up in the introduction.

Response: Description of the infectious zone was included in the introduction.

**Pg. 23 – Results.** In reference to POI by Trinity River reaches, the reviewer suggests that the first two paragraphs be moved to the end of the section because those paragraphs would be better as a summary than an introduction to the Trinity River.

Response: The section was re-written to give specific data by site, and then summarized for overall disease POI for all sampling done in the Trinity River.

**Pg. 28 – Results.** Reviewer questions whether the explanation of historic annual prevalence should be in the methods section rather than the results.

Response: We think it is appropriate to leave the paragraph in the results as we are using it to explain the results in Table 5. We were explaining that histology was the screening method in the earlier years, but that has transitioned to QPCR in recent years. This paragraph is an introduction to the table.

**Pg. 29 – Results.** Reviewer suggests that we move the explanation of bimodal peaks to the discussion.

Response: This is repeated in detail in the discussion (Pg. 38); however we were just explaining briefly that the pattern of this graph is not unusual to see and the discussion goes further to depth about the bimodal peaks and why this occurs.

**Pg. 36 – Discussion.** Reviewer points out that POI for TRH fish while in the Trinity and when they are in the lower Klamath is missing from the discussion.

Response: This data was included in the discussion.

**Pg. 38 – Discussion.** Reviewer finds  $C_T$  values (inversely related to parasite concentration) confusing when referring to  $C_T$  values of natural fish from 2011 and 2012.

Response: Replaced with parasite copy number (concentration) in fish tissues as primary measure, and included CT values for in parenthesis for those more familiar with this unit.

### Reviewer #3

**Pg. 2 – Summary.** Reviewer could like clarification if the CWT fish are adults or juveniles.

Response: The Chinook screened by QPCR are out-migrating juveniles from Iron Gate Hatchery. The summary is an overview of the results; however the fact that we are testing juvenile fish is discussion later in the report on page 5.

**Pg. 2 – Summary.** We stated that there was no manipulated pulse flow in 2012. The reviewer pointed out that there was flow release from Trinity as well as the Klamath River main stem. The reviewer wanted clarification if we are talking about a limited time period.

Response: We included clarifying language to describe a limited time period, and point being made: no large manipulated pulse flow occurred as in 2011. We did add language to the river flow section of the report to state that some water was released from IGD but it was not large and was after our sampling was complete.

**Pg. 12 – Results.** Reviewer thinks the graph is confusing. “I cannot understand how DNA density can be applied to a fish”. Also, suggests that the y-axis of the graph needs to be labeled.

Response: The graph does not represent DNA of a fish, but instead the amount of *C. shasta* parasite DNA found in fish infected with *C. shasta*. The graph title and caption explain that what is being graphed is the Ct value on the y-axis.

**Pg. 18 – Results.** On the POI graph for K2, the reviewer is asking for clarification of *P. minibicornis* on the graph. Was it not sampled or was it not analyzed? Need to distinguish from zero and not sampled.

Response: As explained in the methods, both intestinal (*C. shasta*) and kidney (*P. minibicornis*) tissue are collected from the same fish. Therefore, if fish were collected in a given week for *C. shasta*, it then was also collected for *P. minibicornis*. In the graph, the *P. minibicornis* bars were sub-samples that were tested for Pm. Fish sub-sampled and tested for *P. minibicornis* are summarized in Appendix C.

**Pg. 20 – Results.** The reviewer states that it would be extremely informative to show results from known Trinity River hatchery fish only. Are they becoming infected in the Klamath?

Response: This is addressed in the CWT section of the report. Language changed on page 27 to clarify the POI difference between TRH in the lower Klamath River and TRH fish in the Trinity river itself.

**Pg. 33 – Results.** In reference to mean daily temperature graphs, reviewer suggests that we include more than three years of data.

Response: We do have more years of temperature data, but chose to only include three recent years of data so that prevalence of disease could be compared to last year, and we could show the reader how the environmental conditions affect disease prevalence.

**Pg. 33 – Results.** Reviewer asked if the temperature data acquired from IGH is water temperature at the hatchery or from the river near the hatchery.

Response: The temperature logger for this data is located 150 feet upstream of the Copco road bridge, between the hatchery and the dam. The location was chosen for its lack of other influences on temperature at that point.

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